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SEASONAL DEVELOPMENT OF RIBES[1] IN RELATION TO SPREAD OF CRONARTIUM RIBICOLA IN THE PACIFIC NORTHWEST[2]

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INTRODUCTION

In the western part of North America, *Cronartium ribicola* Fisch. is spread to great distances by the aeciospores, which are carried in a viable condition by air currents far beyond the range of their places of origin.[4 5] The period of maximum production and dispersal of these spores normally is restricted to a relatively short period in the spring. The extent to which they establish the rust on ribes depends to a large degree upon the age and stage of development of the leaves. It has been a common observation in the studies of the rust on ribes in the eastern part of North America[6] that the leaves in the earliest stages of development are remarkably resistant or even immune to infection. Following this early resistance, the leaves quickly reach a stage of maximum susceptibility. As the leaves grow older they harden off and tend to become again resistant, the degree of resistance depending upon the species and the conditions under which the plants are growing. Spaulding, summing up his own observations and those of other investigators of the rust,[7] states:

> The age and relative maturity of the leaf has much to do with its susceptibility. It has been the general experience that Ribes leaves may be overmature and also may be too young to take the disease. Infection does not occur on the leaves of a given species of ribes until they have reached a certain degree of maturity. Leaves produced by buds developing in late summer or fall, even if very small, readily become infected. The different species of Ribes vary much in this regard. *Ribes nigrum* shows a great range in its age of susceptibility, while resistant species become infected only on leaves of a certain maturity. The most favorable stage of growth seems to be about when the leaf attains full size but has not become hardened and leathery as it does later.

ORIGIN OF STUDY PLAN

In the long-distance spread of the rust from pines to ribes in western North America there occurred in 1926 a wide extension northward, whereas the spread was slight in the latitudes in which the aeciospores

[1] The genus name *Ribes*, as well as the common noun "ribes", is used in this paper to include both currants and gooseberries.

[2] Received for publication Dec. 19, 1933; issued August 1934.

[3] The writer was assisted in the collection of the data on which this paper is based by C. J. Nusbaum, J. L. Mielke, C. N. Partington, A. A. McCready, and J. W. Kimmey. Special acknowledgment is due Mr. Nusbaum, who in 1928 had immediate charge of the systematized study in the field of correlation between age of leaf and susceptibility. The writer also wishes to express his thanks to E. P. Meinecke for aid in preparing the final draft of the manuscript.

[4] LACHMUND, H. G. STUDIES OF WHITE PINE BLISTER RUST IN THE WEST. Jour. Forestry 25: 878-881. 1926.

[5] PENNINGTON, L. H. RELATION OF WEATHER CONDITIONS TO THE SPREAD OF WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Jour. Agr. Research 30: 593-607, illus. 1925.

[6] SPAULDING, P. INVESTIGATIONS OF THE WHITE-PINE BLISTER RUST. U.S. Dept. Agr. Bull. 957, 100 pp., illus. 1922. See pp. 45-47.

[7] SPAULDING, P. See p. 45 of reference mentioned in footnote 6.

were produced and relatively negligible in a southern direction. Even in the immediate vicinity of infected pines the infection was light. Figure 1 shows the territory over which the rust was found on ribes

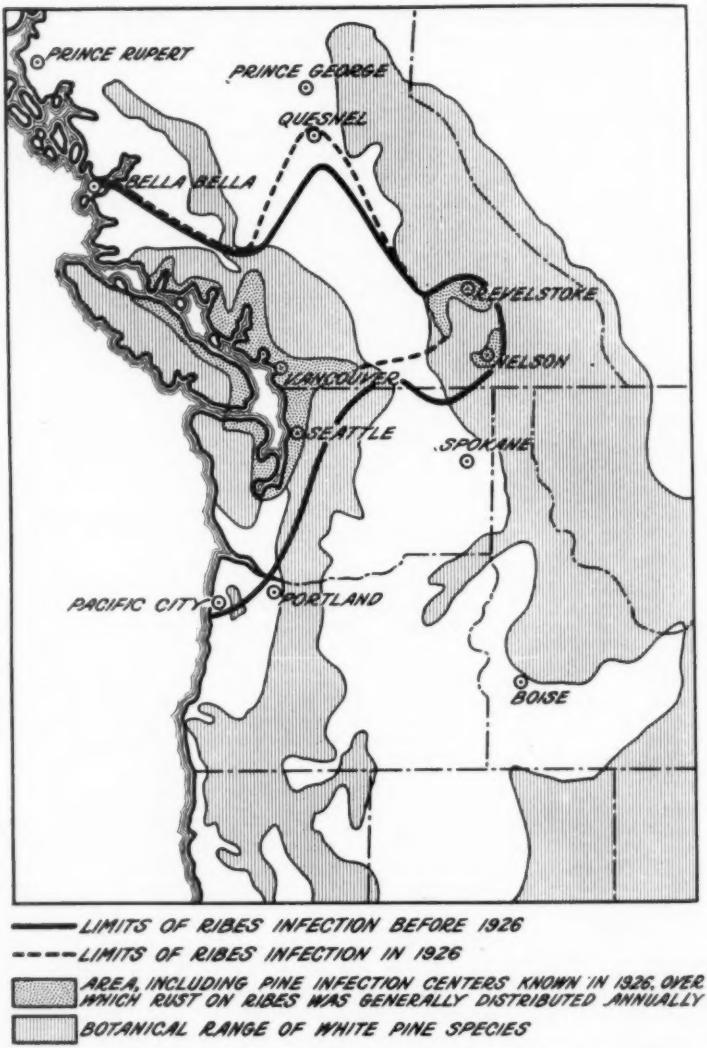

FIGURE 1.—Range of *Cronartium ribicola* on *Ribes* in western North America in 1926 as compared with previous spread.

in 1926, as compared with the limits within which it had been found on these hosts in previous years. As may be seen from this map, the main range of pine infection and source of aeciospores at this time was

confined primarily to the coastal section of southwestern British Columbia and adjacent Washington.

The spring of 1926 opened exceptionally early over the entire region. Ribes leaf development and vegetative activity generally commenced a full 3 weeks earlier than normal. The production of aeciospores by the rust was not correspondingly advanced, however, and the period of maximum spore production occurred at about the usual time, which in this case was about a month after ribes growth in the same localities had commenced. At this time most of the leaves had just attained full size and were succulent and soft without any evidence as yet of hardening. Apparently they were in the most favorable condition to become infected. Aeciospore production was as heavy and moisture and other climatic conditions appeared to be as favorable for infection as in any preceding year. It was difficult, therefore, to explain the light local infection that resulted.

An examination of weather records and actual observations throughout the region indicated that wind and moisture conditions were as favorable for long-distance spread to the south as to the north. Fall reconnaissance for the rust on ribes in 1926 covered a wide area, extending practically from California to the line of the Grand Trunk Pacific Railway in British Columbia and from the Pacific coast into Montana. In general it included the same territory and localities that had been visited in previous years. Eradication of the extremely susceptible cultivated black currant from the region south of the international boundary in 1922, 1923, and 1924 had reduced the opportunities for southward spread of the disease on ribes. The greatest southward distribution up to and including 1926 was not recorded, however, until 1925, a year in which spring in the southern portions of the area was almost a full month later than in 1926. The 1926 scouting included practically all the inspection points and, in general, the identical ribes bushes examined in 1925. Consequently no differences in the kind and quantity of ribes examined could have accounted for the light local infection and the limited southward and wide northward spread observed in 1926 (fig. 1). The writer was therefore forced to the conclusion that in this year the leaves of these hosts were in a more favorable stage of development for infection to the north than in the latitudes of aeciospore production and southward.[8]

Since the beginning of general growth, activity, including ribes leaf development, was as much as a month later in these more northern latitudes than in the localities of aeciospore production, the period of maximum susceptibility of these hosts under western conditions must occur at a very early stage in their seasonal development and be considerably earlier than previous experience with the rust elsewhere would indicate. The results of inoculations in comprehensive susceptibility tests [9] then in progress with various western species of *Ribes* growing under natural conditions in the region had already led to the same conclusion. Since the question bore not only on the

[8] LACHMUND, H. G. WESTERN BLISTER RUST INVESTIGATIONS, 1926. Western White Pine Blister Rust Conference, Portland, Oreg. Rept. Proc. 1926: 19–23. 1926. [Mimeographed.]

[9] These tests were inaugurated in 1924 in the dry belt of British Columbia, an area which is free from white pine but over which the rust had been widely distributed to the cultivated black currant (*Ribes nigrum* L.) by wind-borne aeciospores in 1923. The rust does not overwinter on ribes in this region, and since no white pines occurred within miles of the test plants there was no chance for the disease to become permanently established in the test areas. Furthermore, since the initial infection that might occur from long-distance spread of aeciospores from the outside would be too slight to be of any significance, there seemed to be no probability of complication of the inoculation results.

method of spread but on the general epidemiology of the rust, the writer worked out a plan of study for the purpose of determining the behavior of the most important of these species with regard to their susceptibility from the early stages of development to maturity. The method proposed was, briefly, to mark new leaves periodically until a sufficient basis of age classes of representative leaves of known age was secured, to inoculate all simultaneously, and compare the results. The opportunity to carry out these experiments did not arise until 1928.

SUSCEPTIBILITY OF YOUNGEST LEAVES ON WESTERN RIBES

In the meantime evidence of the early susceptibility of the leaves continued to accumulate from the large-scale susceptibility tests, in which the time of inoculation was advanced to conform more closely with the earliest phenological stages of the ribes. During favorable weather these early inoculations were completely successful. The most susceptible stage seemed to occur during and immediately following the complete unfolding of the leaf, or between 5 and 15 days after the leaf had broken from the bud, and usually before the leaf had attained half of its diameter growth. There was no evidence of immunity even in the very youngest stages. In one case, J. L. Mielke, who assisted the writer in 1927, after covering the tip of one finger with aeciospores, pressed it lightly against a portion of the lower surface of each of several tiny leaves of *Ribes petiolare* Dougl. which were just unfolding from the buds. When these leaves were examined 2 weeks later they had expanded to diameters of 1½ to 2½ inches, and each bore on its under surface the enlarged imprint of the finger tip, reproduced in yellow uredial spots against the green background of the otherwise lightly infected tissues.

The inoculations just described were made on June 5 at Nine Mile Creek on Anarchist Mountain, near Bridesville, British Columbia. At the same time general inoculations were made of several hundred other plants of *Ribes petiolare* and two other species, *R. inerme* Rydb. and *R. lacustre* (Pers.) Poir., by dusting with aeciospores. Good showers fell at the test area on June 7 and 8, after which the weather was clear and dry. The foliage then ranged from leaves just breaking from the bud to those that were nearly mature. The examination was made on June 19, when infection resulting from these inoculations was becoming apparent. On all species the youngest leaves inoculated showed comparatively high susceptibility. The uredia which developed upon them were quite normal and comparable to those produced on older leaves.

In the same year inoculations were made by the dusting of *Ribes petiolare*, *R. inerme*, *R. lacustre*, and *R. viscosissimum* Pursh at test areas near Kelowna and of *R. petiolare* near Summerland, British Columbia, on May 16 and 17, when even the oldest leaves were not yet fully grown and on many bushes ranged from less than one fourth to less than two thirds grown. By June 2 these inoculations had resulted in the appearance of abundant uredial infection, with evidence of the highest degree of susceptibility in the youngest leaves. These results were confirmed by many other data obtained from the inoculations and also from field observations of the degree of infection on ribes as affected by the relation between the time of aeciospore

production and the stage of ribes leaf development in the various pine-infection centers in this and other years.

In most of these cases moisture conditions favorable to infection were virtually confined to the time of observation of the age of the leaves. Wet weather lasting a sufficient time to assure good infection generally accompanied or immediately followed the inoculations and phenological observations. This wet spell was succeeded by clear, dry weather of a type in which general experience with the rust in the West has shown that infection does not occur. Ample evidence of the inhibitory effect upon infection of such dry conditions is also available from eastern investigations.[10] Temperatures were favorable during wet spells, and the incubation period agreed well with the usual.[11] Infection was therefore limited to these moist periods; in other words, it occurred at about the time the observations on the stage of development of the leaves were made, and not at any later time after the leaves were materially older and more developed. Thus the general age-susceptibility relationships shown were conclusive. But they required more precise definition. Such definition could be obtained only through a study of the type projected, i.e., one in which the ages of the leaves at the time of inoculation and infection were positively determined.

GEOGRAPHIC DISTRIBUTION OF RIBES SPECIES TESTED

The four species *Ribes petiolare*, *R. inerme*, *R. viscosissimum*, and *R. lacustre* were selected for study because of their importance in the spread of the rust. These species are not only widespread over most of the range of the rust but are the four main species within the commercial range of *Pinus monticola* Dougl., in the "Inland Empire" region of northern Idaho and adjacent Washington and Montana. Owing to their importance as associates of the pine, inoculation experiments to determine their general susceptibility had been in progress since 1924. A considerable number of plants of known susceptibility were therefore available for the experiments. The areas selected for the study were being used for the fifth season in these susceptibility tests and were located near Kelowna, British Columbia.

Two forms of each species were used, namely "open" and "shade." The plants in the open were entirely exposed to the sun during the greater portion of the day. They were generally short, bushy, and densely branched. The leaves were relatively small and thick and they hardened off at a comparatively early age, usually between 1 and 1½ months after breaking from the buds. The shade plants, on the other hand, were rambling and loosely branched and the leaves were relatively large, thin, and tender, and more sparsely distributed upon the branches. It is questionable whether the term "hardening off" can be properly applied to the leaves of this form. They were still soft, tender, and succulent at ages of 1½ to 2 months, when the leaves of the open forms had become distinctly hard or tough and leathery. Although they toughened to a certain degree thereafter, the process was slow and gradual and never reached a degree comparable with that in the open forms.

The plants of *Ribes viscosissimum* and *R. lacustre* were growing at an elevation of about 1,900 feet, in a rocky canyon drained by a

[10] SPAULDING, P. See pp. 42-44 of reference mentioned in footnote 6.
[11] ——— See p. 40 of reference mentioned in footnote 6.

rapid mountain stream, known as Canyon Creek, located about 12 miles southeast from Kelowna. The plants in the open were situated on a well-drained rocky site, and the shade plants were located nearby, under a moderately dense canopy of mixed conifers and hardwoods, upon a moist soil covered by a thin layer of humus.

The plants of *Ribes inerme* and *R. petiolare* were situated about 5 miles south of Kelowna, in a low undrained swamp where the soil consisted of a mixture of silt and peat covered by a thick mat of slowly decaying grass and other vegetable matter. The soil here was always wet. The open-form plants were exposed to the sun during the entire day. The shade plants were more or less continuously shaded under a moderately dense canopy of swamp hardwoods, such as willows, alders, poplars, etc. The elevation of this area is about 800 feet lower than that at Canyon Creek.

Kelowna lies in a dry belt where rainfall is normally light. Light winter snows are generally succeeded by dry weather in early spring. A short period of rains usually occurs in May or June and the remainder of the growing season is comparatively dry. The rainfall in the Canyon Creek area is normally greater than in the swamp area, owing to the higher elevation and the greater propinquity of the area to the mountains east of Kelowna.

Ten plants of each species and form (shade and open) were selected for the experiments. All except the 10 shade-form plants of *Ribes petiolare* had been proved by the previous infection tests to be susceptible and representative in their reaction to the rust for the particular species and form concerned. A satisfactory group of previously tested plants of the shade form of *R. petiolare* was not available and it was therefore necessary to use untried plants of this form. These plants proved to be normally susceptible and representative in the present experiments.

RIBES PHENOLOGY

Ribes plants are among the first to leaf out in the spring. The first leaves to issue from the buds remain in a cluster at the termination of the previous season's woody growth. After these cluster leaves are out and the oldest are approaching full size, woody growth commences and new leaves develop gradually on the new shoots. Generally these leaves are larger and more uniform in character than the earlier cluster leaves and make up the main body of the foliage developed during the growing season.

There is much variation in the process and character of leaf development among species and among individuals, types of leaves, and individual leaves. The study was started April 15. At that time or shortly afterward, nearly all the buds on both open and shade forms of *Ribes viscosissimum* had broken. In several cases, buds had already begun to open even on branches still buried beneath 2 feet of snow. The leaves just emerging from the buds were folded with the under side out, and were densely covered by glands. The interlocking of these sticky and stalked glands had a tendency to retard the unfolding of the new leaves. Some of the leaves did not completely unfold until as much as 10 or 12 days after breaking from the bud. Production of the cluster leaves was slow. Once formed, they grew rapidly, however, attaining an average diameter of about

1 inch in 10 days. The current season's woody growth began about May 7, when the plants were beginning to blossom. At that time the oldest cluster leaves appeared to be approaching maturity and were 3 inches in diameter. New shoots 4 to 6 inches long were produced, but very few leaves were formed on the young woody growth. These leaves grew rapidly and at maturity were slightly larger than the older cluster leaves.

On *Ribes lacustre* there was considerable variation in the time of opening of individual buds. The first buds commenced to open about April 16. On April 22 about one-third of the buds were still dormant and the remainder were in varying stages of development, from buds swollen but unbroken to buds with leaves 4 or 5 days old. The breaking of the buds continued uniformly for 2 to 3 weeks. The young cluster leaves remained folded until they were about 6 to 8 days old, and were thin, tender, and translucent in appearance until approximately 1 week after they had completely opened, when their texture tended to toughen. At this stage, they had attained a size of one-half to three-fourths of an inch in diameter. The first appearance of the current season's woody growth was noted on May 5, when the blossom buds were beginning to open. At this time the oldest cluster leaves appeared to be nearly mature and were about 1 inch in diameter. The current season's woody growth was most vigorous on the shade-form plants. On the new shoots was produced an abundance of fresh soft-textured leaves that reached a size of 1½ to 2 inches in diameter in 20 days.

On *Ribes inerme* the leaves, as indicated by their size on April 15, must have begun to develop about April 5 in the shade-form plants, a few days ahead of the open form. The cluster leaves broke out of the buds abundantly and grew rapidly, attaining a diameter of about one-half of an inch in a few days. These leaves were completely unfolded in about 6 days and were thin and translucent in appearance. The production of cluster leaves continued for approximately a month, when the growth of the new shoots began and the blossom buds broke. The oldest cluster leaves had then reached a size of about 1½ inches in diameter and appeared to be mature. The growth of new shoots on the shade plants began about a week earlier than on the plants in the open and was more vigorous. Leaves produced on these shoots developed rapidly and soon surpassed the cluster leaves in size. Leaves on the shade plants remained green and succulent, but on the plants in the open they turned yellow and became tough, leathery, and stunted in appearance.

On *Ribes petiolare* leaf development began about April 10 on the shade plants and about April 15 on those in the open. The terminal buds were the first to break and developed more rapidly than the lateral buds, which were mostly fruit buds and produced few leaves. The production of cluster leaves was not heavy. Growth was slow until about 2 weeks after development began, when it became more rapid. There was considerable variation in the time required for the leaves to unfold. Most of them were completely unfolded in 6 days, while others opened partly and then remained in that stage for a time. Those that unfolded rapidly attained a diameter of about 1 inch in 8 days. The new shoots commenced to form on the shade plants on May 10, and on the plants in the open on May 14. Their development was rapid on the shade plants, and the leaves produced upon them

were soft-textured and thin, developing to a diameter of 5 or 6 inches within a relatively short time. On the open-form plants the current season's woody growth was poor and produced relatively thick leaves 2 to 3 inches in diameter. When shoot growth began, the plants were in full blossom and the early cluster leaves had reached a size of about 2 inches in diameter and were mature.

The foregoing phenological observations are summarized in table 1.

TABLE 1.—*Ribes phenology on test areas near Kelowna, British Columbia, 1928*

Species	Form	First buds broke—	Production of cluster leaves stopped—	Blossoming began—	Current season's woody growth began—
R. petiolare	Open	Apr. 15	May 8	May 7	May 14
	Shade	Apr. 10	May 5	May 2	May 10
R. inerme	Open	Apr. 8	Apr. 28	May 4	May 9
	Shade	Apr. 5	do	May 3	May 2
R. viscosissimum	Open	Apr. 12	May 2	May 6	May 7
	Shade	do	do	do	Do.
R. lacustre	Open	Apr. 16	May 7	May 7	May 5
	Shade	do	do	do	Do.

METHODS AND PRELIMINARY OBSERVATIONS

SELECTION OF LEAVES FOR INOCULATION

Every 4 days two new leaves on each plant were marked with paper tags or with threads of different colors so that the leaves of different ages stood out clearly.

The selection of leaves at Canyon Creek began on April 15. At that time buds of *Ribes viscosissimum* were opening, with the leaves just showing from the bud tips, and the buds of *R. lacustre* were swelling, although none of them had broken. It was not until April 21 that the first set of leaves on the latter species was secured.

The selection of leaves on the swamp area began on April 21. On that date nearly all the buds of *Ribes inerme* had opened, and the oldest leaves had accomplished more than half of their total diameter growth. New leaves were being produced rapidly and abundantly. On the shade form of *R. petiolare* most of the terminal buds had already broken and some of the leaves had attained a diameter of as much as 1½ inches. On the open-form plants of this species most of the buds were still unbroken and none of the leaves had yet unfolded. Development on both forms of *R. petiolare* was proceeding slowly.

On all plants of *Ribes viscosissimum*, *R. lacustre*, and *R. petiolare*, leaf selection was begun by tagging buds that were just opening. The number of 1-day-old leaves (those just showing from the bud) that were present on that date was marked on the back of the bud tag. Every fourth day thereafter another examination of each tagged bud was made and the number of new 1-day-old leaves was separately recorded. Where tagged buds ceased to provide sufficient quantities of new leaves, additional buds which had broken later and on which new leaf production was continuing actively, were tagged to make up the difference. Thus a series of leaves of definitely determined ages was provided from which to select for the experiments. The leaves selected were representative of each age class. When each leaf had grown to a size at which it could be handled without injury, it was

separately designated by slipping over it a loose noose of colored thread or by hanging a paper tag on the petiole.

The method of selection described above could not be used for *Ribes inerme*, because this species was phenologically different from the others, the first leaves being well unfolded at the time of the initial visit to the area. Loose nooses of colored threads were slipped over 1-day-old leaves every 4 days from the beginning. This operation required much care to avoid injuring the leaves just emerging from the bud. Each age class was designated by thread of a particular color. Within the age class each leaf was later separately numbered and designated by a paper tag that was either hung on the petiole or attached to the stem close to it.

By these methods a series of 4-day age classes of leaves was developed for each species and form. The selection of leaves was continued until a wide range of age classes was established. A supply of fresh aeciospores was then secured and weather conditions suitable for inoculation were awaited. During this time the periodic 4-day selection of leaves continued. A period of favorable weather began on May 27, and the plants at Canyon Creek were inoculated on May 28 and those of the swamp on May 30.

As already stated, leaf selection was begun on *Ribes viscosissimum* on April 15. The 1-day-old leaves selected on that date were consequently 44 days old on May 28, the date of inoculation at Canyon Creek, and the inoculations included 11 age classes of leaves ranging from 4 to 44 days old. Leaf selection on *R. lacustre* did not begin until April 21. The basis for this species consequently consisted of only 10 age classes, with the oldest leaves 38 days old and the youngest 2 days. On *R. petiolare* and *R. inerme*, at the swamp area, selection was begun on April 21 and inoculations were made on May 30. The basis for each of these species, therefore, consisted of 10 age classes, ranging from 4 to 40 days. These age classes included all stages of leaf development, from leaves just broken from the buds to mature leaves of almost or entirely full size.

MEASUREMENT OF LEAVES

In order to determine the size of the area of leaf surface exposed to the aeciospores, all leaves were measured on the day chosen for the inoculation. For this purpose celluloid disks with concentric circles etched 0.1 inch apart were used. On these circles were etched values [12] for the leaf area in square inches which had been calculated for each species except *Ribes petiolare*. The leaf, still attached to the plant, was placed on the center of one of these scales and three points of a leaf margin were made to coincide with one of the circles of the scale. These scales gave only approximate estimates of actual size but were considered sufficiently accurate for the purposes of comparison of leaf area. Since the scale for *R. petiolare* had not been worked out, the scale for the similarly shaped leaves of *R. lacustre* was used in calculating the leaf area of this species.

[12] These values were determined by the Spokane, Wash., office of the Division of Disease Eradication and Control, Bureau of Entomology and Plant Quarantine, U.S. Department of Agriculture.

INOCULATIONS

Aeciospore material collected on May 15 at Owl Creek, Mile 63, Pacific Great Eastern Railway, British Columbia, was used in the inoculations. On May 28, when the plants of *Ribes viscosissimum* and *R. lacustre* were inoculated, the oldest leaves appeared to have passed maturity. The current season's growth was progressing rapidly, and the fruit was about half formed. On May 30 when the plants of *R. inerme* and *R. petiolare* were inoculated, the current season's growth was vigorous on the shade forms of these species. While the oldest leaves apparently had passed maturity, all growth was still soft-textured. Development on *R. inerme*, open form, was going on slowly. The oldest leaves were yellow and leathery in appearance and some of the plants showed no current season's woody growth. On the open form of *R. petiolare* development was also progressing slowly. The leaves were dark green in color, crisp, and deeply wrinkled, as contrasted with the large, smooth, bright-green and tender leaves on the shade form. The fruit of *R. inerme* was almost fully formed, and on *R. petiolare* it was over half formed.

The inoculations were made in accordance with a simple and effective system which the writer had devised and used successfully for the past 4 years in the general ribes-susceptibility tests. Under overcast skies and when barometric and all other indications pointed to the imminence of rain, the inoculum was applied to the leaves merely by using as a bellows a paper bag filled with freshly sporulating cankers and puffing the spores into the bushes from a short distance. This manner of application assures a fairly even distribution of the spores over the leaves of the bush generally.

Unless there is a fair certainty that the rains will be heavy enough to keep the leaves wet continuously through the day, it is not advisable to make such inoculations until well along in the afternoon, for the leaves dry more slowly then and at night. Plants wet by rains earlier in the day may dry off quickly if the weather clears during the day. Such drying generally cuts the time too short for infection, and kills germinating spores. Inoculations under such conditions are usually complete failures.

Experience in previous inoculations indicated that the weather conditions that attended the inoculation in the present case would assure satisfactory infection. A period of moderate temperatures and rainy weather had begun on May 27, with heavy rains during the night of that day. No rain fell during the day on May 28, when the plants at Canyon Creek were inoculated, but the sky was generally overcast in the afternoon when the inoculum was applied. Rain fell early that night, and the plants remained continually wet under cloudy skies until about noon of May 29, when the weather turned cold and clear.

When the inoculations at the swamp area were made the next day, May 30, the sky was lightly overcast in the early morning. During the day the clouds thickened and general rains began in the early afternoon, continuing intermittently until the following morning, when the sky cleared. The inoculations were made just before these rains began.

TIME OF INFECTION AND INCUBATION PERIOD

After the sky cleared, on the morning of May 31, all plants dried off completely. No further precipitation occurred until the afternoon

of June 1, when a heavy rain fell. This moist period was relatively short as compared with each of those that followed the inoculations. For nearly 2 weeks thereafter the weather was generally unfavorable for infection. June 2 was lightly overcast and windy; June 3, clear and cool; June 4, again lightly overcast and windy; June 5, clear and warm; June 6, clear in the morning, with a little rain in the late afternoon; and June 7, dry in the morning, with light and local showers in the afternoon. From then on it was generally clear and warm until June 13, when another stormy period commenced, during which showers fell on June 13, 15, 16, and 17.

From the character of the weather at the time of the inoculations and later it is evident that in both inoculations the opportunities for infection were practically confined to the hours immediately following the application of the spores. In both cases the leaves remained moist long enough to assure complete germination [13] and infection [14] and then dried off and remained dry for approximately a full day. Certainly very few if any viable spores could have remained after such a wetting and drying. It is not likely, therefore, that any significant infection resulted from tardily germinating spores on the plants at Canyon Creek during the moist period of May 30–31 nor on the plants at the swamp area from the afternoon rain of June 1, but even if such infection had occurred, the difference in time would have been slight.

As previously stated, subsequent weather conditions were distinctly unfavorable until June 13. The first examination of the plants following inoculation was made on June 20. At that time the resultant infection had become fully apparent and the inoculated leaves showed fully matured sporulating uredia, which clearly had made their appearance at least 4 or 5 days earlier, or by June 15 or 16. Consequently it is out of the question that any of the infection could have originated during the moist spell beginning on June 13, for this would have allowed only 2 or 3 days for incubation.

Some of the leaves even showed the beginning of the development of telia. Since telia at this time of the year normally do not appear until at least a week after the uredia, it is more probable that the first uredia made their appearance about June 13. This would allow an incubation period of approximately 2 weeks from the time of the inoculations, which agrees well with the usual period out of doors both in the West and in the East. In western experiments the period has varied from as little as 10 days to as much as 3 weeks, depending on conditions. These studies have indicated that the incubation period is prolonged by cold weather and unfavorable growth conditions which retard the rate of development of the fungus in the host tissues. In eastern experiments the period out of doors has varied from 11 to 18 days and has usually been 13 to 15 days, whereas in the greenhouse it has varied from 7 to 17 days,[15] and has usually been 10 to 12 days.

Neither at Canyon Creek nor in the swamp area was there any difference in the character or apparent age of the rust on the different-aged leaves which might indicate that the youngest leaves became infected later than the others. The uredia on the youngest leaves

[13] Tests with similar inoculum dusted on the surface of a glass of water and kept in shade immediately prior to these and other inoculations had repeatedly given almost complete germination within 8 to 12 hours.
[14] SPAULDING, P. See pp. 37, 41–44 of reference mentioned in footnote 6.
[15] ——— See p. 40 of reference mentioned in footnote 6.

were generally normal and entirely comparable in their character and stage of development with those on older leaves.

In the light of the circumstances and conditions described above there can be no doubt that the infections at each area took place directly after inoculation in each case. The age of the leaf taken at the time of inoculation therefore represents its actual age at the time of infection within a probable margin of not more than a day. This is considered entirely close enough for the purpose of this study.

RECORDING AND COMPUTING

Infection data were taken between June 21 and 30. No new uredia developed during this period. Where infection was light, the number of individual infection spots on each leaf was counted; otherwise, the number of spots was obtained by estimates based on partial counts.

TABLE 2.—*Number of inoculated Ribes leaves of 4 species and their average areas at the time of inoculation and 3 to 4 weeks later, at the end of the experiment*

Species and age classes (in days)	Inoculated leaves of—					
	Open form			Shade form		
	Basic number	Average area		Basic number	Average area	
		At inoculation	At end of experiment		At inoculation	At end of experiment
		Square inches	*Square inches*		*Square inches*	*Square inches*
R. cisocissimum:						
4				18	0.07	1.56
8	20	0.13	0.95	18	.14	1.51
12	19	.49	1.39	12	.30	1.78
16	19	.75	1.62	12	.68	2.26
20	19	.91	1.54	16	.92	1.80
24	16	.65	1.55	15	.84	3.01
28	16	1.00	1.89	15	1.44	2.89
32	20	1.09	1.96	14	1.94	4.06
36	19	1.97	2.91	17	2.55	3.94
40	19	2.23	3.24	20	2.79	3.94
44	18	2.46	3.54	20	3.13	4.97
R. petiolare:						
4	20	.06	2.24	20	.27	6.80
8	19	.30	2.64	19	.90	8.80
12	19	.77	2.46	20	1.41	9.11
16	20	1.09	2.62	20	1.87	9.28
20	20	1.54	3.26	20	3.11	11.21
24	20	1.80	2.96	18	4.95	14.37
28	19	1.37	2.29	19	3.98	10.57
32	20	1.30	2.00	16	3.91	8.52
36	20	.97	1.44	18	2.57	5.12
40	20	.68	.82	18	2.40	3.14
R. inerme:						
4	11	.02	.08	15	.12	1.61
8	15	.15	.37	17	.28	2.00
12	11	.21	.42	16	.43	1.90
16	17	.18	.32	15	.64	1.83
20	19	.21	.33	17	.74	1.61
24	12	.26	.30	17	.84	1.29
28	18	.25	.31	17	1.15	1.83
32	20	.31	.34	20	1.78	2.38
36	19	.29	.31	16	1.90	2.33
40	17	.35	.35	16	1.60	1.71
R. lacustre:						
2	18	.05	.57			
6	16	.08	.90	14	.09	.40
10	19	.31	1.47	18	.23	.43
14	19	.75	1.84	20	.41	.84
18	18	1.21	2.46	20	.53	.85
22	19	1.26	2.35	19	1.13	1.58
26	18	.44	.92	19	.52	.75
30	16	.53	.71	19	.85	.99
34	17	.51	.61	19	.81	.92
38	15	.31	.44	19	.39	[1] .37

[1] Considerable insect damage.

Between inoculation and examination some of the leaves were lost through the activities of chewing insects, and to some extent by the death of the leaves resulting from mishandling during tagging operations. In general, however, there was very little evidence of any damage from this latter cause. All leaves were gathered at the time of examination, pressed, and kept in a permanent file.

Table 2 gives by age classes the basic number of leaves and their average size at inoculation and when the infection data were recorded. The size of these leaves at the time of recording the data is of no importance in the calculation of the degree of infection, since the size of the leaves at the time of inoculation only is used in these calculations. But in connection with the determination of the time required for the leaves to reach full size it provides some valuable comparisons, which are discussed in the following section.

For each age class of each species and form, the number of infection spots and the areas of the leaves exposed to inoculation were totaled. The number of infection spots per square inch of leaf surface was computed by dividing the number of spots by the number of square inches of leaf surface in each case.

RESULTS

Results for each species, shown graphically in figure 2, give no indication of early immunity. In every case there was infection of the 4-day-old leaves. Even in *Ribes lacustre*, where the youngest age class was composed of leaves only 2 days old, the infection compared well with that of any of the older age classes. In certain of the forms, notably *R. petiolare* (open form) and *R. inerme* (shade form) the degree of susceptibility in the youngest age class was close to the maximum attained for that species and form. The degree of infection in these youngest age classes indicates that the leaves must have been susceptible practically at the breaking of the buds.

While there is no evidence of early immunity, the results show that as compared with their peaks of susceptibility the leaves passed through an early period of some relative resistance to the rust. This period is indicated generally to be exceedingly short. The stage of maximum susceptibility was reached in most cases when the leaves were between 4 and 16 days old. In *Ribes petiolare* (open form) the greatest degree of susceptibility occurred in the 4- and 8-day classes, with the peak in the 8-day class. In the shade form of this species, susceptibility rose rapidly to nearly maximum levels in the 8-day class, and the peak came in the 16-day class.

In *Ribes inerme* (open form) the highest point was shown in the 12-day class. In the shade form of this species, the degree of susceptibility rose from a high level in the 4-day class to a peak in the 8-day class. It receded slightly from this peak in the 12-day class and then dropped sharply to a moderate level in the 16- to 28-day classes.

Neither form of *Ribes viscosissimum* showed uniform correlation between age class and susceptibility; it was therefore impossible to determine any peak. In general, there seemed to be a slight upward trend in susceptibility throughout the range of the age classes. The wide variation in degree of susceptibility among individual leaves

of both forms indicated that it would be necessary to use a much larger number of leaves if the true relationship for this species were

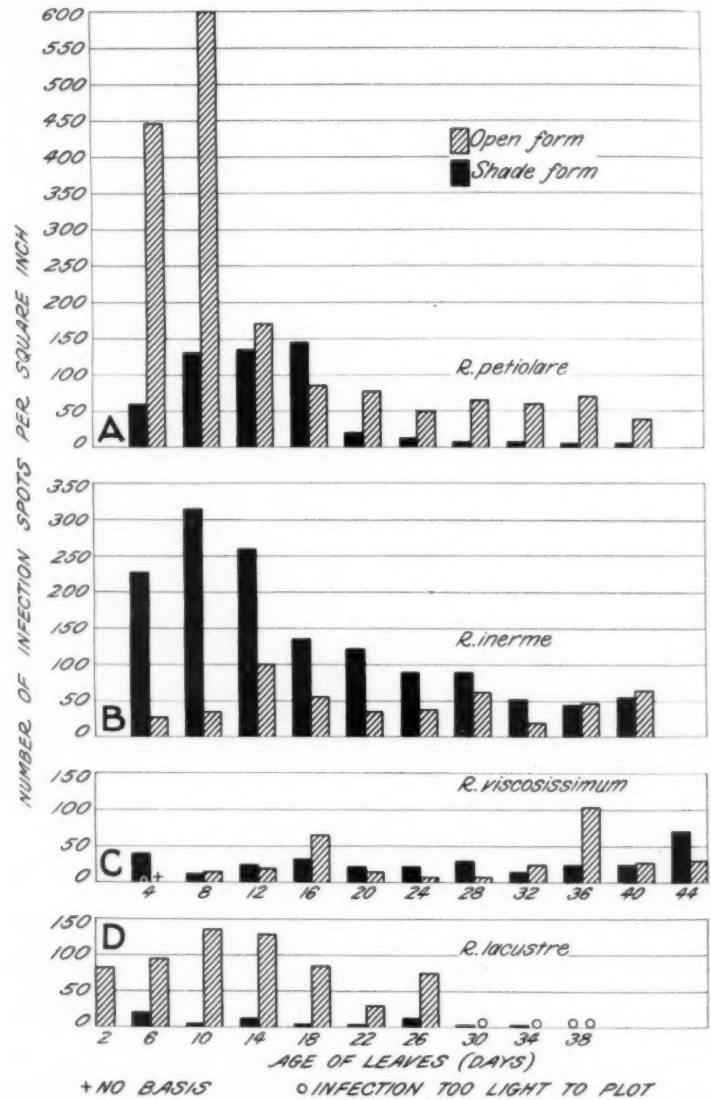

FIGURE 2.—*Cronartium ribicola* on Pacific northwestern *Ribes* species: Diagram showing degree of infection on leaves by age classes.

to be determined. It is evident, however, that both forms are susceptible from a very early age.

For *Ribes lacustre* (open form) susceptibility started at a fairly high level in the 2-day class, rose to its highest levels in the 10- to 14-day classes, and maintained a fairly high level up to and including the 26-day class. Beyond this the degree of infection was negligible. The results for the shade form of this species, like those for *R. viscosissimum*, indicate an insufficient basis of data. The degree of susceptibility in this case was uniformly low. The peak appeared to occur in the 6-day class, although comparatively high degrees of infection occurred also in the 14- to 26-day classes.

The foregoing experiment shows that the stage of maximum susceptibility is generally reached much before the leaves attain full size. Reference to the phenological data will show that in the younger age classes, where susceptibility was highest, the leaves in some cases had not even completely unfolded at the time they reached their maximum susceptibility. In *Ribes petiolare*, most of the leaves unfolded in 6 to 8 days. The highest levels of susceptibility occurred in the open form in the 4- and 8-day classes, and in the shade form susceptibility had reached almost its highest level in the 8-day class. In *R. inerme*, the leaves were completely unfolded in about 6 days. In the shade form of this species, susceptibility was rapidly approaching its highest level in the 4-day class, and the peak was reached in the 8-day class. In *R. lacustre* (open form), the leaves unfolded in about 6 to 8 days. The susceptibility of the leaves of this form was already high when 2 days old.

In Spaulding's use of the term "full size" in his statement concerning the susceptibility of the leaves (p. 93), it is assumed that he refers to "apparent full size" as determined by observations. The development of the leaves to this stage in the present test required in general from 3 weeks to 1 month. In most cases the period of greatest susceptibility, therefore, occurred between about 10 and 20 days before the leaves had reached this stage. This in itself is a decided difference in western as compared with eastern results. But measurements in the present study show that appearances are deceptive and that the time required for the leaves to reach full size may be considerably longer than may be indicated by observation alone. If the time required for the leaves to reach actual full size is taken, the difference between the western and eastern results becomes even more striking.

For the purpose of obtaining data on the rate of leaf growth, a separate basis of 30 leaves just breaking from the buds was selected for each species and form at the beginning of the marking of the leaves for the inoculations. Measurements were made of these leaves every 4 days until the time of the inoculation. These measurements showed very rapid growth in diameter during the first 3 or 4 weeks, after which it slowed down or seemed practically to cease. At this time the leaves would have been classified observationally as having about reached full size. In the leaves used in the present experiments, however, a comparison of the measurements at the time of inoculation with those taken 3 to 4 weeks later, when the plants were examined (table 2), shows that in most cases a very considerable increment in leaf area occurred between these dates in leaves 30 days old or older at the time of the inoculation.

Table 2 indicates a very rapid growth in the youngest age classes, corroborating the results of earlier measurements and observations.

Ribes inerme (open form) shows very little growth for leaves more than 20 days old. Good growth occurred in the shade form of this species, however, in leaves up to 36 days old. Growth was slight in leaves of *R. lacustre* more than 26 days old. The leaves of *R. petiolare* showed extensive growth in the age classes up to 32 days. In leaves of *R. viscosissimum* there was considerable growth even in the 44-day class, and the long period of immaturity indicated in this species may perhaps explain the relatively high susceptibility shown by the oldest leaves.

From the standpoint of the likelihood of infection, the smaller size of the younger leaves must tend to reduce to a certain extent the effect of their higher susceptibility. From table 2, however, it is evident that the rapid increment in leaf area during the earlier stages of development quickly reduces this disparity in size between the younger and older leaves. Furthermore, there is a question whether for spores coming from distant sources the size of the leaf may be ranked on a par with the degree of its susceptibility. But even if this is done and the age classes are compared on the basis of average amount of infection per leaf regardless of size, the general average in the 0- to 16-day classes, where the maximum degree of susceptibility was developed, is still comparatively high.

After the period of maximum susceptibility, resistance developed. In *Ribes petiolare* (open form), susceptibility dropped sharply in the 12-day class, and from the 16-day class on was relatively much below the maximum. In the shade form of this species, the leaves from the 20-day class on were decidedly resistant as compared with the degree of their earlier susceptibility. In *R. inerme* (shade form), there was a sharp reduction of susceptibility in the 16-day class, beyond which susceptibility was gradually reduced still further. For the open form of this species, there was a moderate reduction of susceptibility in the 16-day class, beyond which the average level was fairly even. In *R. lacustre* (open form), there was a moderate decline in susceptibility past the 14-day class and an abrupt development of extreme resistance beyond the 26-day class, coinciding with the rapid hardening of the leaves, which normally occurs about this time in this form of *R. lacustre*. A relative resistance in the shade form of this species seemed to develop about the same time, although the data are so variable as to leave some doubt. Previous inoculation experiments with this species, however, indicate that it generally develops decided resistance after the leaves have reached the age of about 1 month.

A comparison of the results for the different species indicates a higher average degree of susceptibility to infection by aeciospores for *Ribes petiolare* and *R. inerme* than for *R. viscosissimum* and *R. lacustre*. This indication is in conformity with the results [16] of previous susceptibility tests of these species, carried out by the writer or under his direction.

DISCUSSION

The outstanding result of the foregoing investigation is the establishment of the fact that, under western conditions at least, the leaves in the earliest stages of development are highly susceptible to infection by aeciospores. This fact is in direct contradiction to the

[16] In manuscript.

previously accepted belief that such leaves are resistant or even immune to infection.

It is difficult to explain this contradiction in the results obtained under eastern and western conditions. The present study is believed to be the first in which the method has been employed of marking the individual leaves at the beginning of their development and accurately noting their size and condition during the entire course of the experiment so that no confusion could arise as to their ages. The lack of such procedure in previous studies injects an element of uncertainty into the calculations and conclusions. The question arises as to whether young leaves which had developed between inoculation and the time of appearance of the infection and which consequently had received none of the inoculum may have been included in some of the susceptibility comparisons. As for the older leaves, there is uncertainty with regard to their actual age both at the time of inoculation and of examination.

The present study appears also to be the first in which the degrees of susceptibility of the different-aged leaves have been compared upon a basis of equal units of leaf area exposed to infection. Obviously the younger leaves being smaller would receive a smaller amount of inoculum per leaf and might show less infection though actually having a higher degree of susceptibility than the older ones.

The results of the western and eastern experiments are still less comparable because of differences in the kind of inoculum used. In the former the inoculations were made entirely with aeciospores, whereas in the latter many of the inoculations were made with urediospores. Nothing is known exactly of the relative capacity of aeciospores and urediospores to infect leaves of different ages. However, general observations of the seasonal development of the rust on ribes, beginning with the appearance of the initial infection resulting from aeciospores and extending through the usual successive waves of uredial intensification until final defoliation of the bushes, have shown that the leaves remain susceptible to urediospores over long periods. In favorable seasons and with susceptible species, such as *Ribes petiolare*, it is common to find on the same leaves 4 or 5 generations of the rust, each heavier than the preceding one. On numerous species of *Ribes* in the West, heavy uredial intensification has been observed under natural conditions as late as October; even the oldest leaves have shown a high degree of susceptibility.

In the present study, on both shade and open forms of *Ribes petiolare* and on the shade form of *Ribes inerme* leaves of the same age classes as had shown the highest relative resistance in the aeciospore inoculations became heavily infected by uredial intensification during the season, although the quantities of urediospores developed on the plants and responsible for the intensification were much smaller than the quantities of aeciospores applied in the inoculations. In the general susceptibility tests the initial infection resulting from the inoculations has regularly been light as compared with the subsequent waves of infection that have developed on the bushes from uredial intensification. These observations suggest not only that the urediospores are more effective than the aeciospores but also that the leaves remain susceptible to them for much longer periods.

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Finally, in the eastern experiments there was a large variation in the method and time of application of the inoculum and in the treatment of the plants after inoculation,[17] whereas in the western experiments the following method was invariably used: Aeciospores from paper bags containing sporulating cankers were puffed into the bushes out of doors, on late afternoons or during cloudy weather, in the spring immediately before rains. In a few cases the rains were falling during the inoculation and in a few others the rain failed to materialize. In the latter cases the plants were reinoculated later by the same method when conditions again became favorable. In general, the intensity of the inoculation, i.e., the age and quantity of the inoculum used, was fairly comparable throughout.

After discounting all differences between the eastern and western studies there still remains a considerable range in which they are fairly comparable. This comparability applies in the present case, particularly for leaves just breaking or broken from the buds at the time of inoculation in eastern experiments in which aeciospores were used and the plants kept under almost daily observation thereafter. In such cases there could be little doubt as to the age of these particular leaves. In the main, these eastern studies indicate such leaves to be immune or highly resistant.[18]

In certain experiments by Snell reported by Spaulding,[19] however, results seem to have been in rather close agreement with western results. The account of these experiments as given by Spaulding is as follows:

> Snell * * * made inoculations out of doors with spores from unopened aecia on April 30, 1918. He inoculated opening buds of *Ribes glandulosum* by carefully inserting a knife so as not to injure the leaflets and inserting the spores between the folds of the leaves. The largest leaves were 3 cm broad, the smallest 3 to 5 mm long. It was very rainy, so there was plenty of moisture. No infection was visible on May 15, but on May 22 heavy infection was present on all the leaves inoculated. The leaves on this date ranged from nearly full size to those just opening. The infection was heaviest on the largest leaves inoculated and decreased to a light infection on the smallest. The check plants were healthy. There are two possible factors which might have delayed the infection a week longer than usual. These are cool temperature and the immaturity of the leaves. The experience of the writer leads to the belief that the latter was the principal factor involved in this case. Later Snell found natural infection on leaves of *Ribes vulgare* that were only 12 mm wide.

In the light of the western results and of the fact that there was plenty of moisture for these eastern inoculations, the writer is inclined to believe that the infection took place at once and that the cool weather was responsible for the incubation period being longer than normal.

More recently Hahn,[20] [21] in greenhouse experiments with numerous species and varieties of *Ribes*, including the species used in the present study, has obtained results for the youngest leaves tending to corroborate other results obtained in the East. His inoculations, however, were made primarily with urediospores. Of the species involved in the present test, among others, he states: [22]

[17] SPAULDING, P. See p. 41 of reference mentioned in footnote 6.
[18] ——— See pp. 45–46 of reference mentioned in footnote 6.
[19] ——— See p. 46 of reference mentioned in footnote 6.
[20] HAHN, G. G. THE INOCULATION OF PACIFIC NORTHWESTERN RIBES WITH CRONARTIUM RIBICOLA AND C. OCCIDENTALE. Jour. Agr. Research 37: 663–683, illus. 1928. See pp. 666–679.
[21] ——— A PHYSIOLOGICAL METHOD OF DISTINGUISHING CRONARTIUM RIBICOLA AND C. OCCIDENTALE IN THE UREDINIAL STAGES. Jour. Agr. Research 40: 105–120. 1930. See p. 107.
[22] See p. 679 of reference mentioned in footnote 20.

In the case of very young leaves inoculated just below the terminal or growing-shoot tip, infections of the resistant type were encountered with *Ribes viscosissimum*, *R. petiolare*, *R. howellii*, *R. laxiflorum*, *R. lacustre*, and *R. sanguineum*. Undersized uredinia associated with flecks or larger necrotic areas on the immature leaves of these species gave place to normal uredinia on further developed, fully expanded leaves.

In the present study infection of the resistant type was not encountered on the younger leaves to any greater extent than on older leaves, and the uredia even on the youngest leaves were generally normal and entirely comparable with those of other susceptible leaves.

In correspondence with the writer, Hahn has expressed the belief that there is probably greater similarity in respect to relationships between leaf age and susceptibility in rust development under eastern and western conditions than appears to be the case from the results thus far reported. With regard to the most susceptible stage for infection, his own results, although obtained with unrediospores and without definite determination of the ages of the leaves, appear to be in closer agreement with the writer's than do those of some others. In correspondence concerning the point, Hahn states that he secured optimum urediospore infection upon leaves which had fully expanded but had not commenced to harden. This might include a range in age from perhaps 30 days, depending on when his leaves hardened, down to as little as 6 days, and the lower portion of this range at least would overlap the range of maximum susceptibility indicated in the present studies.

Hahn believes that the term "full size" as used by Spaulding in relation to the stage of leaf development embraces in reality leaves of a relatively younger and more undeveloped stage than might be understood by the term. However, Spaulding states with reference to inoculations by Snell [23] that leaflets of *Ribes glandulosum* ranging to 3 cm in breadth on April 30, 1918, ranged to nearly full size on May 22. Since the oldest of these leaves were more than 23 days old, it is clear that Spaulding's definition of apparent "full size" coincides closely with the writer's. Consequently, and in view of the repeated evidence of immunity or high resistance of the youngest leaves in eastern experiments and of their high susceptibility in the western experiments, the writer is forced to conclude that there is a difference in the time of susceptibility of the leaves under eastern and western conditions, and that after breaking from the bud the leaves are susceptible at an earlier age in the West. Possibly this may be due to a more gradual advent of spring growth activity in the West, resulting in a more prolonged development of the leaves in the buds, or to some similar cause. This is a question which must be left to future studies.

The early age of susceptibility of ribes leaves shown in the present studies places a new light upon considerations of the spread and general epidemiology of the disease. The results substantiate the earlier conclusions of the writer with respect to the northward spread of the disease in 1926.

General observations of the rust in western North America by the writer and his associates from 1922 to 1933 have shown that the time of aeciospore production is less subject to influence by variations in seasonal conditions than is the commencement of growth activity in ribes. When spring is early, ribes growth activity is generally ad-

[23] SPAULDING, P. See p. 46 of reference mentioned in footnote 6.

vanced to a greater degree than is aeciospore production. This throws ribes leaf development past the stage of maximum susceptibility during the period of maximum aeciospore production at similar elevations in the same latitude. Therefore, in the localities of aeciospore production and in localities at lower elevations and at more southern latitudes, the leaves are in a relatively unfavorable condition to become infected, while at higher elevations and in more northern latitudes they are passing through the stages of maximum susceptibility. Thus when spring is early, the differences in time of leaf susceptibility and aeciospore production have the effect of intensifying the spread to higher elevations and more northern latitudes and in lessening the spread to the south and to lower elevations.

Conversely, when spring is late ribes leaf development is retarded to a relatively greater degree than is aeciospore production. Under these conditions, ribes leaves at higher elevations and in more northern latitudes may not yet have broken from the buds at the time aeciospore dispersal is in progress. Late springs, therefore, will tend to lessen the spread to the north and to high elevations and to favor the spread to the south and to lower elevations.

Aside from the effect of this variation in general spring conditions, the direction of spread will be powerfully influenced by unequal regional variations from the normal. In general, any conditions which advance spring north and retard it south of the aecial source will extend the northward and southward ranges of synchronization of maximum leaf susceptibility with maximum sporulation at the aecial source, and vice versa. The most favorable conditions for long northward spread will occur when spring is abnormally early in the region to the north and abnormally late at the aecial source. The most unfavorable conditions for such spread will come about when these conditions are reversed. The most favorable conditions for southward spread will occur when spring is abnormally late in the regions to the south and abnormally early at the aecial source. The least favorable conditions for southward spread will occur when these particular conditions are reversed. Conditions will be most favorable for a wide spread in all directions when spring is normal at the aecial source and in the same latitudes, abnormally early to the north, and abnormally late to the south. The situation will be least favorable for such a spread when spring is early at the aecial source and to the south, throwing ribes leaf development beyond the stage of maximum susceptibility at the time of maximum sporulation, and when it is late north of the aecial source, retarding the breaking of the buds in this direction until after the aeciospores have been dispersed; in other words, under a set of conditions in which maximum aecial sporulation would fail to synchronize with the development of the maximum susceptibility of ribes leaves in any direction. Such a combination of conditions, however, is likely to occur very infrequently, as ribes leaves will generally be in a favorable stage for infection in some direction within the range of long-distance aeciospore dispersal in any season; so there usually is a wide spread in some direction every year.

Another conclusion to be drawn from these data is that the greatest southward spread may generally be expected in the higher elevations. Under normal conditions, the more southern the latitude, the earlier is the leaf development of ribes. Given a source of aeciospores at a particular elevation and latitude, the most favorable ribes leaf development

will usually coincide fairly well with maximum aeciospore production at this point. At similar elevations in more southern latitudes, the leaves will be in a more advanced stage of development. The lines most favorable for spread in a southward direction, therefore, will follow those along which ribes leaves are at favorable stages of development or along a gradual incline into higher elevations the farther south the observer travels. Conversely, the greatest northward spread will occur toward lower elevations and from aeciospore sources at the highest elevations.

SUMMARY

Under western conditions *Cronartium ribicola* each spring spreads over great distances to ribes plants by means of wind-borne aeciospores. The degree, extent, and direction of this spread are largely governed by the stage of susceptibility of ribes leaves at the time the main body of aeciospores is produced. Other investigators have found that under eastern conditions ribes leaves are immune or resistant in the beginning and that they reach their most susceptible stage when they have attained full size but have not yet hardened off; the data here presented show that under western conditions the most susceptible stage is much earlier.

Preliminary observations showed that ribes leaves generally require about 6 days to unfold and that they apparently attain full size in about 3 weeks to 1 month after breaking from the buds. Hardening and toughening of the tissues do not begin until after this stage has been reached.

For the four most important species of western ribes, inoculations were made in the field on leaves ranging in age from 2 to 44 days. Resultant infection had fully developed 3 weeks after the inoculations, evidently having made its appearance from 4 to 7 days before the first examination. The degree of infection shown by the youngest classes indicated that the leaves were susceptible from the time they emerged from the buds. The periods of highest susceptibility lay in general between the ages of 2 and 16 days, after which susceptibility declined. The decline was very sharp in some cases and more gradual in others.

The most favorable period for spread of the disease by aeciospores occurs when the period of maximum spore dispersal from a given center synchronizes with that in which the largest proportion of the leaves lying within the long-distance range of the spore showers are in their most susceptible stages. The time of ribes leaf development seems to be more affected by variations in seasonal conditions than is the time of aeciospore production. An early spring at the aecial source advances the development of the ribes leaf until it is beyond the stage of maximum susceptibility during the period of maximum aeciospore production. This restricts infection at the aecial source, and lessens spread to lower elevations and to the south, where leaf development is still more advanced, but favors spread to higher elevations and to the north, where spring is naturally later and ribes are in more favorable stages of development. A late spring has the opposite effect, lessening spread northward and to higher elevations, where the leaves may not yet have broken from the buds, and favoring spread to the south and to lower elevations.

Variations in the time of spring within any region also exert a strong influence on the direction of spread. In general, any conditions advancing spring to the north and retarding it to the south of the aecial source extend the northward and southward ranges of synchronization of maximum leaf susceptibility with maximum sporulation at the aecial source and consequently extend the range of favorable conditions for long-distance spread, and vice versa. Thus, early spring in the north and late spring at the aecial source favor northward spread.

Likewise, the farthest northward spread may be expected from aeciospore sources at high elevations to ribes at low elevations in the north, and the longest southward spread may be expected from aeciospore sources at low elevations to ribes at high elevations in the south.

SOME IMPROVEMENTS IN AUTO-IRRIGATOR APPARATUS[1]

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INTRODUCTION

Soil moisture is a variable in plant experiments that scientific investigators have long been endeavoring to control. The two most practical schemes that have been used for this purpose are the constant-weight and the constant-capillary-tension methods.

By the constant-weight method moisture control is attained through the periodical addition of sufficient water to bring the soil container up to a standard weight. This method involves certain disadvantages that are hard to overcome. If the water is added by hand, considerable routine work is involved; if the process is automatic, as Steinberg (*8*, *9*)[3] has recently made it the equipment is rather expensive where many units are needed. The intermittent supply of moisture and the inaccuracy arising from neglecting the changing plant weight are other disadvantages.

The constant-capillary-tension method, which at present seems to be the most promising system for controlling soil moisture, makes use of some modified form of the Livingston auto-irrigator. By this method the water is continuously supplied to the soil through a porous wall that is in contact with free water. Irrigators of this type have been in use for over a quarter of a century, and the chief difficulties connected with their use have been (1) unreliability because of poor capillary contact between cup and soil; (2) the difficulty of obtaining sufficient water-supplying surface without limiting the space for soil and roots; and (3) air leaks.

The first of these troubles was partially avoided by using cone-shaped porous cells instead of cylindrical cups. The next big advance came when Korneff (*3*), Richards (*6*), and Wilson (*10*), working independently, developed the double-walled auto-irrigator pot. By the use of this newer unit the first difficulty aforementioned may be completely eliminated, and with many plants and soil-moisture contents the second difficulty will not occur. However, the third difficulty, that of excluding air from the water reservoir, must still be recognized and properly cared for, especially if the irrigators are to function for long periods without attention.

It is the purpose of the present paper to describe some further improvements in the construction and arrangement of auto-irrigator apparatus and to discuss briefly the factors that determine the precision with which soil moisture may be controlled.

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[1] The writers wish to express their appreciation to Dr. B. L. Richards, of the Utah Agricultural Experiment Station, for his interest and active cooperation in this work.

[2] Reference is made by number (italic) to Literature Cited, p. 120.

ONE-PIECE DOUBLE-WALLED POTS

Since the water supplied to the porous wall of the auto-irrigator is maintained at a pressure less than that of the atmosphere, there is the problem of keeping air from leaking into the supply-water reservoir. The first double-walled pots used (*3*, *6*, *10*) were made in two pieces and sealed together. As this seal proved to be a source of considerable trouble, the writers attempted to construct a one-piece double-walled pot. A local clay, the ceramic and capillary properties of which were known from previous use, was mixed with water to form a "slip". From wood patterns, plaster casts of the correct size for the inner and outer walls of the pot were made up (fig. 1, *A*, *B*). A 1-inch hole was drilled near the top of the larger cast so as to form the spout. To make a pot these casts are filled with slip and allowed to stand until, when the contents are removed, there remains a layer of wet clay about one-fourth of an inch thick on the inside of the cast. This layer is allowed to dry until it is just firm enough to

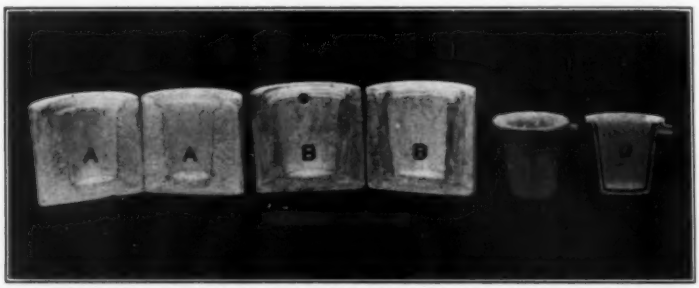

FIGURE 1.—Plaster-of-paris casts for one-piece double-walled pots: *A*, *A*, Cross sections of inner walls *B*, *B*, cross sections of outer walls; *C*, completed pot; *D*, cross section of completed pot.

handle. The inner wall is then removed from its cast and placed inside the outer wall, the flanges connecting the two being covered with a small amount of slip and pressed together. After being further dried, the outer cast is removed from the pot, and a ½-inch hole is made in the end of the spout. The pot is then slowly dried for several days, heated in a warming oven at 100° C. for a few hours, and fired at 950° in an electric furnace built for the purpose.

Figure 1, *C*, shows the form of the finished product. At *D*, the cross section of an unfired pot shows the nature of the water cavity between the walls and the connection with the spout.

As might be expected, some trouble was experienced with breakage during drying. However, when the inner and outer walls were sealed together as soon as they could be handled and when the pots were dried slowly in a rack that permitted free circulation of air on all sides, it was found that the breakage could be reduced to a fairly low percentage.

WATER CONNECTIONS FOR GROUP OPERATION

The form of pot shown in figure 1 was chosen because it permits simple and trouble-free connections with the water supply and is suitable for greenhouse operation on a large scale. Figure 2 shows

one method of connection used. A horizontal ¾-inch pipe, shown in cross section at *a*, extending the length of the greenhouse bench, has threaded in its sides at the desired intervals short lengths of ⅜-inch pipe (*b*). A tube (*c*), which may or may not contain a mercury barostat, connects with a supply tank (*d*), in which the water level is automatically maintained by a ball cock. At some convenient place along the supply pipe (*a*) a vertical ⅜-inch pipe connects with the inverted bottle (*e*), which is fitted with a small air-removal tube (*f*). When *f* is connected with an aspirator vacuum pump the water flows up tube *c*, filling the water chambers in the porous pots and finally rising to the top of *e*. The cock in *f* is then closed.

The tension of the water in *a* is determined by the combined head of the water and the mercury in the tube *c*. The tension of the water in the pores and on the surface of the inner wall of the pot has this same value. Thus, by controlling the tension of the water that comes in contact with the soil, the capillary tension of the soil water is controlled.[4]

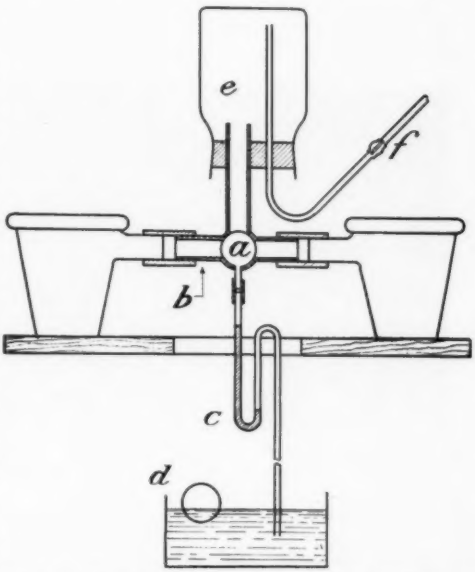

FIGURE 2.—Diagram of water-supply and distributing system for multiple operation of auto-irrigator pots: *a*, Cross section of horizontal ¾-inch pipe; *b*, ⅜-inch pipe; *c*, tube; *d*, water tank; *e*, inverted bottle; *f*, air-removal tube.

Because of the size of the hole in the pot spout (three-eighths of an inch) and the other conducting tubes, any air leaking into the system will quickly move to the chamber *e*, and as long as water is visible in this chamber it is certain that all the pots are filled with water and are functioning properly. By the proper manipulation of pots and rubber stoppers, it is possible to remove pots from a system or to add new ones to it without serious disturbance.

With proper horizontal interconnections of the ¾-inch pipe it would be possible to operate by means of one supply tube any number or configuration of pots at any one tension. It would, however, be necessary to have a separate distribution pipe, *A*, and supply pipe, *C*, for each different tension required. If the number of pots on a system is sufficient to justify the expense, it would not be difficult to set up an arrangement that would automatically remove the air from *E*.

[4] RICHARDS, L. A., and GARDNER, W. CAPILLARY ACTION IN SOIL AND THE USE OF POROUS CERAMIC WARE IN THE MEASUREMENT OF SOIL MOISTURE. (Unpublished manuscript.)

SEPARATE OPERATION

If it is desired to have the pots function as individual units that can be moved about, there are several ways in which this can be accomplished (*10*). The arrangement shown in figure 3 is convenient when a considerable number of pots are involved. The lower end of the mercury barostat dips into water that can be held in long narrow trays and automatically supplied to them. Whether the pot is functioning properly or not can be quickly determined by observing whether the space, *A*, below the rubber stopper, is filled with water. Here again the large spout hole makes it certain that any air leaking into the system will collect at *a*. With this arrangement the pots may be moved around freely, and the barostat may be left out of the supply reservoir as long as the turned-up end (*b*) contains water. The height of mercury in the barostat determines the capillary tension in the pot.

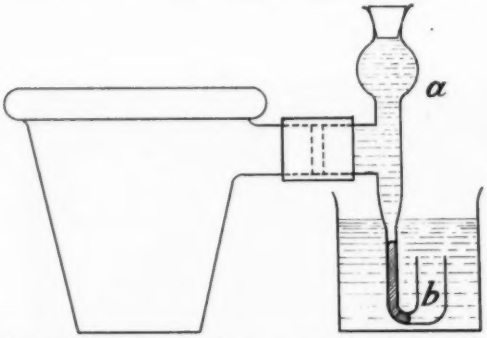

FIGURE 3.—Diagram of pot with pressure-control barostat for use when each pot is to be an individual unit: *a*, Space below rubber stopper; *b*, turned-up end of barostat.

OTHER FORMS OF AUTO-IRRIGATORS

A porous water-supplying surface in the form of a horizontal flat plate is distinctly advantageous for certain work. Seedlings grown for transplanting could be cared for easily by this means and would be less susceptible to some of the damping-off fungi that are particularly active where the soil surface is kept wet. As an illustration of a flat-plate auto-irrigator there is shown in figure 4, a photograph of a germination experiment conducted by one of the writers in 1926.[5] A known number of kernels of Dicklow wheat were spread on each porous plate and covered over with one-half inch of air-dried Greenville soil. Each unit was covered with a glass cage to prevent evaporation. The capillary tension of the soil water on each plate was, from left to right, respectively, 0, 10, 20, and 30 cm of mercury. The experiment was conducted in a dark thermostat at a temperature of $17.2° \pm 0.1°$ C.

From this and other photographs for different sets of values of the capillary tension it was possible to make the following generalization: For Dicklow wheat in Greenville soil at a temperature of 17.2° C., the percentage germination and rate of growth increases as the capillary tension decreases, until, as the capillary tension approaches zero, the soil becomes saturated and the supply of oxygen in the soil solution is insufficient for germination.

[5] RICHARDS, L. A. CAPILLARY POTENTIAL AND ITS POSSIBLE USEFULNESS TO PLANT INVESTIGATORS. Master's thesis, Utah Agricultural College. 1927. (Unpublished manuscript.)

FACTORS AFFECTING PRECISION OF CONTROL

For the purposes of discussion the two extreme ways in which porous-clay auto-irrigators may function to control the capillary tension of soil are designated by the terms "static" and "dynamic".

Under ideal static conditions no part of the soil is far from a moisture-supplying surface, the capillary conductivity of the soil is high (*7*),[6] and no evaporation from the soil is permitted; consequently a small difference in the capillary tension produces a flow sufficient to supply the needs of the plant roots, and the capillary tension of the soil at all points on the same level will be nearly equal to the value of the capillary tension of the water in the porous wall at that level. Under these conditions the amount of flow in the soil has but a small effect on the capillary tension at the plant roots.

Under dynamic control, however, the capillary tension at the plant roots is largely determined by the flow. If the porous water-supply surface is small and at some distance from the roots and if the capillary

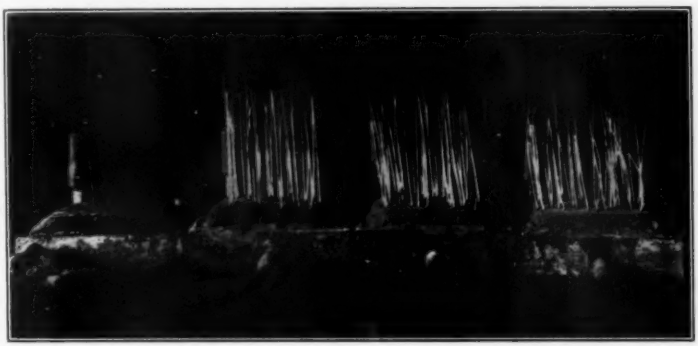

FIGURE 4.—Auto-irrigator cells, with flat horizontal porous surface, showing wheat seedlings 10 days after planting, when the capillary tension of the soil was, from left to right, respectively, 0, 10, 20, and 30 cm of mercury.

conductivity of the porous wall and soil is small, then the capillary tension at the roots will differ considerably from the value at the porous wall and will change rapidly with the rates of evaporation and transpiration. Thus it is evident that when precise moisture control is desired the conditions for the static type of control should be fulfilled as nearly as possible.

Soils differ widely in their capillary water-conducting properties; when conditions permit, a soil with a high capillary conductivity should be chosen. Also, it should be kept in mind that the higher the tension at which the cell operates the lower will be the capillary conductivity, and the operation will tend toward the dynamic type of control.

Even though conditions may be such that the moisture control is definitely of the dynamic type, good results may be obtained. Korneff (*4*), Bordas and Mathieu (*1*, *2*), and Mathieu (*5*), at the agricultural experiment station at Avignon, France, have for several years been using auto-irrigator apparatus for outside field work and have ob-

[6] RICHARDS, L. A., and GARDNER, W. See footnote 4.

tained some exceedingly interesting results. Their auto-irrigators were in the form of fired clay cylinders 7 cm in diameter and 60 cm long. These were connected end to end with rubber tubing and were laid in trenches 20 to 150 cm deep and 200 to 250 cm apart. The results from 2-year experiments (*2*) with potatoes, corn, and melons, may be summarized as follows.

The control plots were surface-irrigated twice a week and received about 6.5 times as much water as the auto-irrigated plots. The crops on the auto-irrigated plots gave heavier yield, matured earlier, and were less susceptible to disease. During the summer the auto-irrigated plots were cultivated only after rains and grew practically no weeds. The account of costs and income is shown in table 1.

TABLE 1.—*Cost and income data for subirrigated and surface-irrigated plots planted with potatoes, corn, and melons, at Avignon, France*

Item	Subirrigated plots	Surface-irrigated plots
Expenses	$10.49	$17.01
Receipts	69.07	38.39
Profit	58.58	21.38
Advantage	37.20	

These results show the practical usefulness of the method.

SUMMARY

The advantages and disadvantages of the constant-weight and constant-capillary-tension methods for controlling soil moisture are discussed, and directions for the construction and operation of one-piece double-walled auto-irrigator pots are given. The types of soil-moisture control that may be expected from porous auto-irrigator units are briefly analyzed.

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ANTHESIS, POLLINATION, AND FERTILIZATION IN SORGHUM [1]

By J. C. STEPHENS, *assistant agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and J. R. QUINBY, *superintendent, Substation No. 12, Texas Agricultural Experiment Station*

INTRODUCTION

Some knowledge of the blooming habits of a species is necessary before artificial hybridization can be successfully accomplished, and reports on the blooming habits of cultivated crops frequently are based on observations made preliminary to hybridizations undertaken for crop improvement or for other purposes. Several of the various phases of blooming in sorghum (*Sorghum vulgare* Pers.) have been described, but most of the observations have been made in India on Indian varieties. American varieties are largely of African origin and differ in some respects from the typical Indian varieties.

Since descriptions of the sorghum inflorescence have been published, they need not be repeated in full. It may be pointed out, however, that the spikelets usually occur in pairs. One spikelet of the pair is sessile and contains one perfect flower; the other spikelet is pediceled, and the flower is either staminate or the glumes are empty. The terminal sessile spikelet of a seed branch is usually accompanied by two pediceled spikelets. A number of introductions from India have two perfect flowers in nearly all sessile spikelets, but none of these are grown in the United States except occasionally for experimental purposes. Cron (*3*)[2] and Karper (*6*) have reported that more than one seed sometimes develops in a spikelet in some of the American varieties but these instances are relatively rare, and the spikelet contains normally but one fertile flower.

The observations reported in this paper were made at Texas Substation No. 12, Chillicothe, Tex., during the period from 1928 to 1932.

Climatic conditions at Chillicothe are representative of a large section of the area of the United States where grain sorghums are produced, but undoubtedly the time of blooming differs somewhat in different localities and varies from season to season or at different periods of the same season in any one locality.

ANTHESIS

TIME OF DAY FLOWERS BLOOM

It has been generally observed by those working with sorghums in the United States that the major portion of blooming takes place in the night or during the early morning hours. Ball (*2*) and Robbins (*13*) state that most of the flowers open in the early morning and very few during the day. Nafziger (*10*) says that "stamens do not emerge

[1] Received for publication Nov. 28, 1933; issued August 1934. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Texas Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 136.

during the heat of the day, but emerge freely during the night." In a greenhouse at Washington, D.C., Vinall (*16*) was able to "collect viable pollen at 8:30 or 9 a.m., even tho the sorghum flowers opened in greatest numbers much earlier in the morning."

Patel and Patel (*11*), in reporting observations at Surat, India, on the varieties commonly grown in the Gujarat (region surrounding Surat), found no blooming previous to 4 a.m., and on damp or chilly nights blooming started from 7 to 8:30 a.m. Ramanathan (*12, p. 1*) at Bellary, India, some 400 miles south of Surat and much farther from the coast, states: "When the temperature is high during the nights, the spikelets commence opening at about 1 a.m. When the nights are chill the opening is delayed even up to 8 a.m." He also says: "Stray flowers are often observed to open as late as 4 p.m." At Nagpur, India, Graham (*5*) made hourly counts of the flowers blooming in 16 panicles on six successive nights in March 1914, and other counts in 6 panicles in August 1915. He summarizes his findings as follows (*5, p. 202*):

> During the months of March and April, the larger number of flowers opened between the hours of 1 a.m. and 4 a.m., but stray flowers opened as early as 11 p.m. In June and July, the flowers opened mainly between 3:30 a.m. and 4:30 a.m., but on rainy nights occasional flowers opened as early as 8:30 p.m., and this continued till 4 p.m., the following afternoon.

More recently, Ayyangar and Rao (*1, p. 449*), working at Coimbatore, India, recorded the blooming of six varieties at 2-hour intervals and state that these varieties—

> commence opening their flowers at midnight. The commencement of the anthesis is not uniform but varies from 12 midnight to 2 a.m. generally. The opening continues till 8 a.m. usually and may get prolonged to 10 a.m. at the most. In the case of a red-grained, compact-headed variety of *S. durra* type, the opening was as late as 4 a.m., stopping at 8 a.m. * * * Wet weather delays flowering, and after flowering, delays the opening of anthers.

Table 1 presents a detailed record of bloom counts made at Chillicothe in two panicles of Spur feterita in 1928 and one each in 1929 and 1930. The counts were made every hour throughout each 24-hour period, but since only occasional blooming occurred between the hours from 12 to 9 p.m. and 10 to 12 a.m., the counts for the hours within each of these periods are lumped together. The maximum number of flowers opening in 1 hour each night is shown in boldface type. This mode or peak of blooming varied from night to night, but it appeared between 1 and 2 a.m. more often than during all other 1-hour periods. Of the total number of Spur feterita flowers opening, 72.1 percent opened between midnight and 4 a.m.

Blooming counts of Spur feterita, as well as counts of Blackhull kafir, Standard Yellow milo, and Chinese Amber sorgo, obtained in a similar manner, are summarized in table 2. The blooming counts published by Graham (*5*) and by Ayyangar and Rao (*1*) are shown also. Among the American varieties, it may be seen that kafir blooms somewhat earlier than feterita and that Chinese Amber sorgo blooms at about the same time as feterita, but 73.4 percent of the milo flowers opened between 4 and 7 a.m. That there is a distinct varietal difference in hour of maximum blooming also appears in the data taken from Ayyangar and Rao.

TABLE 1.—*Number of flowers blooming each hour in 4 panicles of Spur feterita during each day of their blooming periods*

Day of blooming (no.)	Year	Number of flowers opening at indicated hours															Percentage of total in panicle
		P.M.				A.M.[1]											
		12-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-12	
1	1928									29	3						2.7
										26	4	1	1				1.9
	1929											1					.1
	1930								8	**52**		4		2			2.9
2	1928		6	**77**	65	45	36	17	44	22	5	1					26.6
			1	57	51	**87**	75	15	12	20	9	3					19.7
	1929					1	4	6	**59**	7	1	2					6.0
	1930					28	**268**	156	74	25	12	5	2	1			24.9
3	1928	1			15	24	**129**	41	27	21	56	52	6	4		1	31.5
			1		21	16	38	67	50	69	**95**	19	7	3	3	1	23.3
	1929				1	32	**118**	58	17	10	4	1				1	18.1
	1930	3	7	9	52	**175**	142	132	79	45	28	39	2	3	2	1	31.4
4	1928	3			10	23	**74**	56	35	71	4	19	19			1	26.3
		3			7	33	**153**	106	80	61	4	7	4				27.3
	1929	1			8	**203**	151	27	8	5	3	4	6				31.1
	1930	1			3	13	81	93	**108**	38	26	17	1				16.6
5	1928				11	27	**54**	23	10	5	1	3					11.2
			3	3	34	85	**126**	58	13	9	2	5		2	1		20.3
	1929	1			3	57	**156**	60	16	5	2	2			1	1	22.7
	1930		3	4	24	60	**155**	35	10	2	2	1	3	2	2		13.2
6	1928						3	1	1	8	4	1	2				1.7
			1		5	17	**26**	19	18	13	7	5	8	1	1		7.2
	1929			1	3	29	**40**	5	4	5	39	5	2				9.9
	1930			1	12	18	25	41	**55**	27	12	9	6	4	3	3	9.4
7	1928																
		4															.2
	1929				1	20	**35**	28	15	18	17	4				1	10.4
	1930	7	4	4	2	1	1	2	4	3	2	1	1	1		2	1.5
8	1928																
	1929						1	1	6	1	5	5	1				1.5
	1930																
9	1928																
	1929					1			2	1							.3
	1930																

[1] The a.m. counts are those which followed p.m. counts of the same night.

The fluctuation of maximum blooming in Spur feterita from hour to hour on successive nights, as shown by the boldface numbers in table 1, was common to all varieties. Graham's counts (table 2), assuming he used the same variety both years, show the peak of blooming to have been about 2 hours later in August 1915 than in March 1914. In each of the reports from India previously cited, the observation is made that external factors, such as damp weather and low temperature, influence the time of blooming. The number of nights with the mode of blooming at designated hours for each of the Chillicothe varieties is shown in table 3.

DURATION OF BLOOMING IN A PANICLE

Graham (*5*) found that the length of time required for all the hermaphrodite flowers of a panicle to open varied with the size of the inflorescence and averaged about 7 days. Ayyangar and Rao (*1*) give 8 or occasionally 9 days as the maximum time, with the peak of blooming between the third and sixth days. The varieties observed by Patel and Patel (*11*) completed blooming in from 9 to 12 days. Table 4 gives the percentage of total blooming of hermaphrodite flowers on each day of the blooming period for the panicles observed in detail at Chillicothe and for the panicles observed by Patel and Patel.

TABLE 2.—*Summary of hourly bloom counts in varieties investigated at Chillicothe, Tex., and those reported in India*

Location, date, authority, and varieties	Number of panicles	Number of flowers opening at indicated hours														
		P.M.				A.M.[1]										
		12-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-12
Chillicothe, Tex., 1928-30:																
Spur feterita	4	24	26	156	328	995	1,891	1,047	755	598	347	216	71	23	13	12
Blackhull kafir	3	46	171	734	1,557	1,576	1,167	526	453	557	427	157	107	62	36	16
Standard Yellow milo	2	26		5	5	2	13	24	180	1,110	1,319	844	553	249	94	33
Chinese Amber sorgo	3	12		2	23	193	620	518	373	339	228	196	92	30	13	5
India:																
Graham (*5*):																
March 1914	16				358	1,096	1,267	1,233	1,093	797	521	443	439	214	117	46
August 1915	6	[2] 96	[3] 99			[4] 154			1,170	[5] 330	[6] 81			[7] 96		
Ayyangar and Rao (*1*):																
Sen-cholam	1							[8] 12		[9] 2,117		[10] 14				
Chinna-manjal	1							[8] 3,039		[9] 89		[10] 30		[11] 6		
Chitrai-vellai	1					[12] 944		[8] 2,019		[9] 424		[10] 95		[11] 27		
Peria-manjal	1					[12] 1,620		[8] 1,693		[9] 238		[10] 13				
Talaivirichan	1					[12] 3,235		[8] 850		[9] 17		[10] 5				
Irungu	1					[12] 1,511		[8] 365		[9] 14		[10] 9		[11] 2		

[1] The a.m. counts are those that followed p.m. counts of the same night.
[2] Flowers opened between 7:30 and 9:30 p.m.
[3] Flowers opened between 9:30 and 10:30 p.m.
[4] Flowers opened between 10:30 p.m. and 3 a.m.
[5] Flowers opened between 4 and 4:30 a.m.
[6] Flowers opened between 4:30 and 7:30 a.m.
[7] Flowers opened between 7:30 a.m. and 4 p.m.
[8] Flowers opened between 2 and 4 a.m.
[9] Flowers opened between 4 and 6 a.m.
[10] Flowers opened between 6 and 8 a.m.
[11] Flowers opened between 8 and 10 a.m.
[12] Flowers opened between 12 and 2 a.m.

The Chillicothe varieties completed blooming in from 6 to 9 days. Probably a panicle seldom completes blooming in the Chillicothe environment in less than 6 days, but more than 9 days is required under certain conditions. When temperatures are relatively low, as late in the season, it sometimes takes 14 or 15 days for large-headed types like Ajax or Bishop to complete blooming. Under natural field conditions, with sufficient soil moisture available, blooming is most rapid at temperatures prevailing in August.

In the panicles of American varieties maximum blooming occurred on the third or fourth day. Among the Surat varieties maximum blooming was on the fourth or sixth day, but because of varietal differences or environmental conditions these varieties required a longer period than the American types for the full course of flowering. In general, the peak of blooming among hermaphrodite flowers was at about the middle of the panicle and on a day or two before the middle day of the full period of blooming.

TABLE 3.—*Hours in which the greatest number of sorghum flowers opened at Chillicothe, Tex.*

Variety	Years	Panicles	Number of nights peak of blooming occurred at indicated hour [1]								
			P.M.		A.M.						
			10–11	11–12	12–1	1–2	2–3	3–4	4–5	5–6	6–7
	Number	*Number*									
Spur feterita	3	4	1		3	12		3	1	1	
Blackhull kafir	2	3		7	3	3		1	1	1	
Standard Yellow milo	2	2							4	4	2
Chinese Amber sorgo	2	3				5	2	3	2	1	

[1] No nights were included unless the total bloom exceeded 50 flowers.

TABLE 4.—*Percentage of hermaphrodite flowers that opened each day throughout the blooming period*

Location, variety, and authority	Percentage of flowers opening on indicated day										
	1	2	3	4	5	6	7	8	9	10	11
Chillicothe, Tex.:											
Spur feterita:											
1928	2.7	26.6	31.5	26.3	11.2	1.7					
	1.9	19.7	23.3	27.3	20.3	7.2	0.2				
1929	.1	6.0	18.1	31.1	22.7	9.9	10.4	1.5	0.3		
1930	2.9	24.9	31.4	16.6	13.2	9.4	1.5				
Blackhull kafir:											
1928	13.0	21.5	34.0	21.0	9.3	1.0	.1	.1			
	6.3	22.6	34.3	23.8	11.0	1.6	.1	.1			
1929	.7	4.4	18.8	36.2	27.7	8.5	2.9	.8			
Standard Yellow milo:											
1928	3.5	28.7	35.9	28.7	3.1	.1					
1929	1.3	8.2	17.0	34.4	25.3	10.8	3.0				
Chinese Amber sorgo:											
1928	.6	12.2	32.5	30.8	20.2	3.7					
	8.7	29.0	32.6	23.2	5.8	.7					
1929	2.5	9.6	24.9	39.7	15.9	6.2	1.2				
India (Patel and Patel (*11*)):											
Althan Deshi 6	9.7	5.8	8.8	31.1	13.2	15.4	2.8	7.8	5.4		
	.3	1.1	5.4	31.9	13.1	14.9	20.8	5.5	3.9	1.5	1.6
Budh Perio 9	.2	2.7	6.4	13.3	9.1	24.2	18.6	10.4	9.9	4.7	.3
Chapti 8	.2	.7	3.2	12.5	24.1	25.1	17.3	12.9	3.7	.2	
Sholapuri 1	1.5	9.6	17.0	23.8	23.5	14.7	7.0	2.6	.2		

At Chillicothe staminate flowers began blooming from 2 to 4 days later than the hermaphrodite flowers and required about the same number of days for complete blooming. This observation is in agreement with that of Patel and Patel.

ORDER OF BLOOMING IN A PANICLE

It is readily apparent that it would be practically impossible to obtain and record the exact time of opening of each of the individual spikelets in a normal-sized sorghum head, for at the peak of blooming from 100 to more than 400 spikelets may open within an hour. Consequently, a diagrammatic drawing of one seed branch on each successive node from the apex to the base of a Spur feterita head was made; and, insofar as possible, a record was kept on each spikelet of the exact time the glumes began to open, reached full spread, began to close, and completed closing. The time at which the anthers became pendent and the time at which they dehisced were noted. There were six seed branches which required 7 days for complete blooming, and the time at which the glumes began to spread was missed on only a few of the approximately 300 spikelets. Additional observations were made on several panicles of Spur feterita and on several panicles of other varieties.

Usually the first flower to open in a sorghum inflorescence is the terminal or second flower of the uppermost panicle branch. Blooming continues downward in a fairly regular manner, each flower following the one above. There are many exceptions in which a flower or a relatively large group of flowers may open previous to some of those above or may be delayed until after a variable number of lower flowers have bloomed, but such cases are exceptions to the normal course of successive blooming. In a general way, the flowers in a horizontal plane across the panicle open at the same time; that is, when a lower seed branch is overlapping the one above, flowers which are on the same level in the two branches usually bloom at about the same time.

BLOOMING OF THE INDIVIDUAL FLOWER

For the normal Spur feterita flowers observed the average time required from the beginning of glume spread to other phases of flowering was as follows:

To fully spread, 7 minutes; range, 3 to 13 minutes.
To beginning of closing, 20 minutes; range, 13 to 36 minutes.
To fully closed, 2 hours and 7 minutes; range, 29 minutes to 4 hours.

Ayyangar and Rao (*1*) give 20 minutes as the time required for a typical flower to open fully and 45 minutes from opening to closing, but state that some varieties take longer for the full process. Graham (*5*) says the glumes remain open 2 to 3 hours and Ramanathan (*12*), 3 or 4 hours.

Flowers of the common American varieties of sorghum open rapidly. As the glumes begin to spread the anthers and stigmas push out, and the anthers assume a pendent position at the time the glumes are fully spread or shortly before. The anthers usually dehisce at about the time they become pendent, but the moment of dehiscence varies considerably. Sometimes the split occurs before the anthers begin to tilt and often they hang pendent for a short time before dehiscence.

The splitting is usually sudden, and if the anther is tilted or pendent a small cloud of pollen is released.

Under normal conditions neither the stigmas nor the anthers in the American varieties are visible until a few minutes before the glumes begin their rapid spreading caused by the swelling of the lodicules, but there are occasional exceptions. In reporting on 6 Indian varieties, Main (*9*) states that in 1 variety the style appears 48 hours before the stamens but that in an Egyptian variety the stamens protrude first. Ayyangar and Rao (*1, p. 451*) made the following statement:

> Körnicke observes that the stamens and stigmas protrude simultaneously. Kirchner, Graham [1916], Ramanathan [1924], and Patels [1929] say that the stigmas come out first, sometimes as early as 15 to 36 hours before the appearance of the stamens. The last, however, record that in certain Egyptian varieties grown at Surat, the order is reversed. At Coimbatore, the anthers appear first in a column enclosing the two stigmatic branches which are adpressed to their inner faces.

Most of the sorghums introduced into the United States have been grown at the Chillicothe Station for at least 1 or 2 years. Some observations have been made on blooming in many of these varieties, and it appears that protrusion of essential flower parts before the glumes spread in some varieties and not in others is the result of morphological differences. The glumes of varieties in which the anthers or stigmas protrude are usually short and broad or the anthers or stigmas are relatively large and the developing flower forces the tips apart before the lodicules begin to swell.

Exposure of the stigmas before the anthers dehisce of course subjects the flowers to possible pollination from other plants. The extent of natural cross-pollination between different plants has been variously reported from none, by Main (*9*), to as much as 50 percent, by Ball (*2*). Karper and Conner (*7*) found 6 percent in milo, and Sieglinger (*14*) found a little over 5 percent. At the Chillicothe station there is always cross-pollination from adjacent rows if the varieties bloom at the same time. Some cross-pollination between plants is generally recognized, but the rather extensive discussion in the literature of whether the individual flower is usually self-pollinated or usually pollinated by another flower of the same panicle is, from the breeding standpoint, largely academic, for the progeny of the head is the same in either case.

INFLUENCE OF LIGHT, TEMPERATURE, AND HUMIDITY ON BLOOMING

Experimental Methods

Since the bulk of the sorghum flowers open within a relatively short time, and since the peak of the blooming period varies somewhat from day to day, the natural assumption is that some environmental or external factors must affect the time of anthesis. No equipment was available for finely controlled experiments, but feterita plants were grown in tubs in 1930 and 1931 and placed in a photographic dark room for general observations on the influence of light, temperature, and humidity. The heads produced on these plants were small, but on the control plants growing in tubs outside the dark room the panicles bloomed normally. The data collected during the blooming periods, such as the number of flowers opening each hour and the

temperature and relative humidity in the room, are too numerous to be shown concisely in tabular form and therefore are presented in graphs.

The records for one of the controls are given in figure 1. Normal blooming is shown to occur in the period of darkness, roughly from 6 p.m. to 6 a.m., and at the time of the 24-hour day when the temperature is relatively low and the relative humidity high. In this case the temperature and relative humidity readings are those taken regularly morning, noon, and night for the meteorological records of the station, but when panicles were observed in the dark room temperature and humidity readings were made in the room at 3-hour intervals.

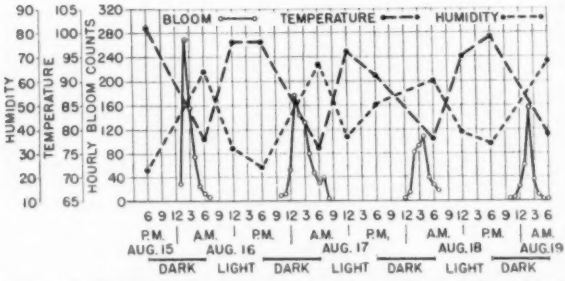

FIGURE 1.—Flowering, temperature, and relative-humidity records for 4 days of the blooming period of a feterita plant grown in a tub and kept outside as a control to represent normal field-grown plants.

INFLUENCE OF LIGHT

A second plant was allowed to start blooming outside under natural conditions. Flowering was normal, and in the early morning of September 2 the bulk of opening occurred between 1 and 3 o'clock. This plant was moved into the dark room before sunrise and kept in darkness until 6 p.m., when a 150-watt electric light was turned on and allowed to burn until 6 a.m. Thereafter the plant was kept in darkness during the natural day and under light during the natural night until blooming was completed. A change in the normal rhythm or periodicity of blooming in this plant and the time required for the change are shown in figure 2. During the second natural night (Sept. 2–3), with the plant, under light, a peak of flowering occurred between 9 p.m. and 1 a.m., roughly 24 hours after the previous peak, when the plant was outside in normal surroundings. However, the next peak of blooming developed approximately 12 instead of 24 hours later. Thus, the new rhythm was established within a period of about 36 hours. Thereafter the bulk of flowering occurred in dark-

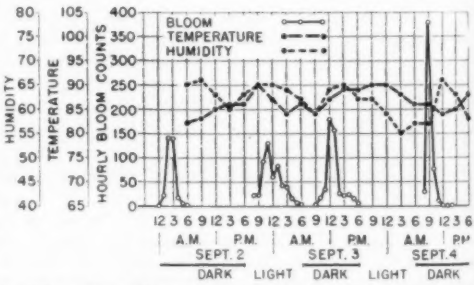

FIGURE 2.—Flowering, temperature, and relative-humidity records for part of the blooming period of a feterita plant grown in a tub, after blooming had started the plant was moved (at 5:30 a.m.) to a dark room where the normal periods of light and darkness were reversed.

ness during the natural day. Temperature and humidity were held as constant as possible while this plant was in the dark room; both varied somewhat but considerably less than usually occurs outside. In reversing the normal plant rhythm, the influence of light (or absence of light) on the time that sorghum flowers bloom was established. Probably the effect is that light acts to inhibit temporarily or to retard the opening of flowers.

Influence of Temperature

During its blooming period one plant grown in a tub was kept outside under a 150-watt electric light at night and in the dark room during the day. Under these conditions the natural relations of light and darkness to temperature and humidity were reversed. Normally, during the period of daylight the temperature is relatively high and the humidity low, and during the period of darkness temperature is low and humidity high. The blooming record of this plant is shown in figure 3. It is obvious that the effect of temperature or humidity influenced the rhythm of flowering in this inflorescence. A major portion of the flowers opened during darkness, but there was a rather large amount of blooming in light and some flowers opened every hour.

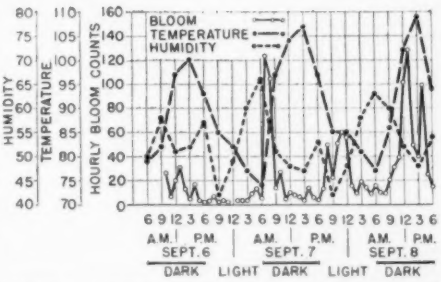
BLOOM
TEMPERATURE
HUMIDITY
HUMIDITY
TEMPERATURE
HOURLY BLOOM COUNTS
80 75 70 65 60 55 50 45 40
110 105 100 95 90 85 80 75 70
160 140 120 100 80 60 40 20 0
6 9 12 3 6 9 12 3 6 9 12 3 6 9 12 3 6 9 12 3 6
A.M. P.M. A.M. P.M. A.M. P.M.
SEPT. 6 SEPT. 7 SEPT. 8
DARK LIGHT DARK LIGHT DARK

FIGURE 3.—Flowering, temperature, and relative-humidity records for part of the blooming period of a feterita plant kept outside under a 150-watt electric light at night and in a dark room during the day.

For another plant kept in the dark room (fig. 4) an attempt was made to hold humidity constant and to reverse temperature in its normal relation to light. Humidity varied considerably but less than temperature. High points occurred during darkness but there was a decided scattering of blooming, and a goodly number of flowers opened during the periods of light.

Influence of Humidity

With temperature relatively constant, humidity was changed to the reverse of its normal relation with light (fig. 5). The plant held in the dark room under these conditions bloomed in a fairly normal manner.

Discussion

Though these experiments lacked somewhat in refinement, they show rather definitely that light (or absence of light) and temperature exert the principal external influences in creating and maintaining the periodicity of blooming in sorghums. Experience with the sorghum crop tends to verify the results. With cloudless days and normal temperatures in July, August, and early September, it is usually difficult to get viable pollen for cross-pollinations except quite early in the morning, and sorghum flowers are seldom seen blooming in the daytime. On the other hand, after a sudden drop in temperature or late

in the fall, when night temperatures are low, blooming is decidedly delayed. During a cool period in October, when minimum night temperatures ranged from 36° to 46° F., Spur feterita did not begin to bloom until 8:30 to 9:30 a.m.

The effect of humidity within ordinary ranges seems to be negligible. Graham (*5*), Patel and Patel (*11*), and Ayyangar and Rao (*1*) all infer

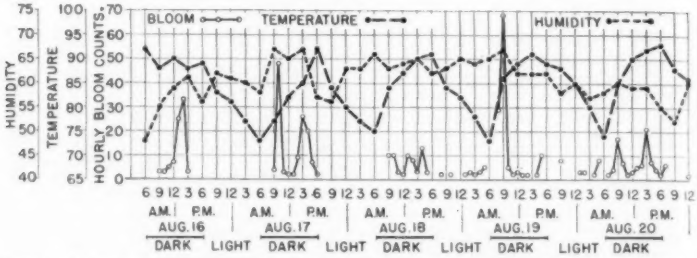

FIGURE 4.—Flowering, temperature, and relative-humidity records for part of the blooming period of a feterita plant kept in a dark room, where the temperature was reversed in its normal relation to light.

that the moisture conditions of the air influence the time of blooming. It should be noted, however, that light and temperature are both usually abnormal during damp or rainy weather, and effects ascribed to humidity may have been largely the results of the other factors.

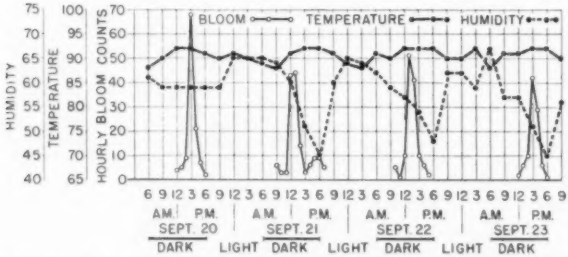

FIGURE 5.—Flowering, temperature, and relative-humidity records for part of the blooming period of a feterita plant kept in a dark room, where the relative humidity was reversed in its normal relation to light.

In a preliminary report of their extensive studies in anthokinetics, Goldsmith and Hafenrichter (*4*) say:

> Floral movements may be initiated by changes in either temperature or light, or by the simultaneous alteration of both. Humidity is not effective in causing or hastening movement, but it does influence the duration of the open condition in many hemeranths and nyctanths.

These investigators did not study grasses.

EFFECT OF WIND ON BLOOMING

At Chillicothe there is enough air movement practically all the time, day and night, to at least slightly disturb the sorghum panicles, and the presence of some wind is considered a normal condition. According to Leighty and Sando (*8*), touching or jarring a wheat head is likely to hasten the opening of flowers. By checking against surrounding plants, it was found that handling the sorghum panicles did not alter the time or duration of blooming under ordinary field conditions. In the course of making many controlled pollinations, however, it has been observed that blooming is delayed on occasional

very quiet nights. On two such nights Spur feterita pollen was needed at 3:30 a.m., but no flowers had bloomed although normally the panicles should have been in or past full bloom. Shaking the stalk or rubbing the panicle vigorously caused a mass of flowers to open within 10 to 30 minutes. Similar shaking or rubbing on windy nights gave no increased blooming. The exact length of time that blooming may be delayed by the absence of the usual air movement was not determined.

POLLINATION AND FERTILIZATION

POLLEN

The normal pollen grains were counted in two Spur feterita anthers. Each anther contained almost 5,000 pollen grains, or approximately 15,000 to the flower. The mean number of hermaphrodite flowers in four panicles of feterita, the blooming records of which are given in table 1, was a little over 1,600. An inflorescence of such size should, therefore, produce about 24,000,000 pollen grains, excluding those of the staminate flowers. The seed on a large but not exceptional F_1 head of Blackhull kafir × hegari numbered 6,727. If pollen was equally abundant in the anthers, this single sorghum panicle produced over 100,000,000 grains of pollen in its hermaphrodite flowers.

Some fresh pollen germinated on a glass slide in about 20 minutes after dehiscence of the anthers. The time elapsing before the germination of other pollen grains ranged up to 1 hour after collection. By that time practically all the pollen had collapsed. Patel and Patel (*11, p. 19*) say:

> With fresh pollen from complete flowers, germination in distilled water took place after 40 minutes; in 5 per cent. glucose solution after 30 minutes; in 10 per cent. glucose solution after 25 minutes; and in 15 per cent. glucose solution in the few cases where germination took place at all, in about 15 minutes.

These authors report that pollen from staminate flowers germinates a little more rapidly in dilute solutions than pollen from complete flowers.

Pollen from staminate flowers of Ajax was placed on the stigmas of emasculated flowers of two other varieties. In both cases seed was set, showing that the pollen was functional. Patel and Patel (*11*) secured similar results.

On five different occasions emasculated flowers were pollinated to determine the length of time pollen ordinarily remains functional. The pollen was collected from flowers which had bloomed within a period of 15 minutes, and pollinations were started 30 minutes later. These pollinations were made at ½-hour intervals for 3 hours and at hourly intervals thereafter. From 10 to 30 emasculated flowers were liberally pollinated at each ½-hour or hourly period in each of the five trials. Averaging all trials, in which a total of over 600 flowers were pollinated, the percentage of seed set from these pollinations was found to be as follows:

Time (hours):	*Percentage of seed set*
½	60
1	37
1½	26
2	20
2½	20
3	10
4	3
5 and above	0

These results indicate that pollen deteriorates very rapidly at Chillicothe. Patel and Patel (*11*) say that "Pollen collected on one morning is capable of germination for at least 30 hours, and cross-pollination was successful after this time * * *." At Chillicothe pollen kept in glass vials and in glassine bags, under three different storage conditions, namely, in an ordinary household refrigerator, in the shade at room temperature, or in the sun for 24, 30, and 48 hours, under each storage condition, did not produce seed in a single instance.

TIME STIGMAS ARE RECEPTIVE

The stigmas of a sorghum flower are receptive to pollen for some time before blooming and for a longer time after blooming. Of the feterita flowers pollinated approximately 24 hours before opening, over 50 percent set seed; of those pollinated 48 hours before opening, almost 50 percent set seed. Since, under Chillicothe conditions, the pollen is viable for only a short time, the stigmas were receptive at the time of pollination or shortly thereafter.

If pollination has not taken place, the extruded stigmas of sorghum flowers remain feathery and fresh-looking for several days after the glumes have closed, even in midsummer, and flowers pollinated 8 days after blooming have produced seed. With the advent of cooler weather in the fall the stigmas remain fresh for a longer period, and successful pollinations were made in early October on Blackhull kafir stigmas 16 days after blooming. The long receptiveness of the stigma is of value in hybridizing sorghums because pollinations may be delayed until all the flowers emasculated for a particular cross have bloomed. When the bulk-emasculation method (*15*) is used and all the flowers in a panicle are to be pollinated, this possible delay in pollination is especially useful, for a week or more frequently is required for the completion of blooming throughout the inflorescence. On the other hand, receptive stigmas are always susceptible to natural cross-pollination, and if care is not taken, bags which have been placed on heads to prevent natural crossing may be broken or removed while unpollinated stigmas are still receptive.

TIME BETWEEN POLLINATION AND FERTILIZATION

A number of Spur feterita flowers were carefully pollinated with fresh pollen. Ovules of these flowers were collected at 2-hour intervals and prepared for examination to determine whether fertilization had taken place. The material was embedded in paraffin, sectioned, and stained with Heidenhain's iron haematoxylin.

No divisions of egg or endosperm nuclei were found in slides prepared from ovules collected 6 hours or less from the time of pollination. In one ovule of the 8-hour period the endosperm nucleus had divided at least twice, showing that the gametes had united some time before, but the egg nucleus could not be found; in another slide the egg had not been fertilized. Of two ovules collected after 10 hours, fertilization had occurred in one and not in the other. In all the ovules examined 12 hours after pollination, fertilization had taken place, and in some the endosperm nucleus had divided several times.

Some fertilization takes place in sorghums in from 8 to 10 hours after pollination, and probably the peak of fertilization occurs at about this time. Undoubtedly the time varies, but insufficient

material was examined to warrant the statement that fertilization cannot occur in less than 6 hours or that it always takes place within 12 hours.

SUMMARY

In a number of panicles of each of four varieties of sorghum, counts were made of the hermaphrodite flowers that opened each hour during the full course of blooming, from apex to base of the inflorescence. For all panicles of each variety, the average peak of flowering occurred at the following time: In Blackhull kafir, between 11 p.m. and 1 a.m.; in Spur feterita and in Chinese Amber sorgo, between 1 and 2 a.m.; in Standard Yellow milo, between 5 and 6 a.m. A relatively small proportion of flowers opened before 10 p.m. or after 8 a.m., but there were no hours in which flowers were never found opening. In all varieties the hour of maximum blooming fluctuated from night to night.

On the panicles observed in detail, from 6 to 9 days was required for the blooming of all hermaphrodite flowers. Late in the season as much as 15 days was required for the panicles of some varieties to bloom. The largest number of flowers to open in a 24-hour period was noted when blooming was at approximately the middle of the panicle. Staminate flowers began opening from 2 to 4 days later than hermaphrodite flowers.

The first flowering in the panicle was at the apex, and this was followed by a fairly regular downward progression. Often isolated flowers or groups of flowers opened slightly out of order.

From the time the glumes first began to spread, individual spikelets of Spur feterita averaged 7 minutes to become fully open, 20 minutes to begin closing, and 2 hours and 7 minutes to become completely closed. The anthers usually became pendent at the time the glumes were fully spread or before. They generally dehisced when assuming the pendent position, but the moment of dehiscence varied considerably.

When a plant was kept under a light at night and in a dark room during the day, the normal rhythm or periodicity of blooming was reversed in approximately 36 hours. Changes in temperature modified the rhythm. Relative humidity apparently did not influence the time of blooming.

In the absence of any wind to disturb the panicle, blooming was retarded for an undetermined length of time. When flowers had not begun to open after the usual time for heavy blooming, vigorous agitation of the panicle or stalk produced an abundance of open flowers within 10 to 30 minutes.

There were approximately 5,000 pollen grains in each of two anthers of Spur feterita examined. Assuming 15,000 as the mean number of grains per flower, it follows that four panicles of Spur feterita produced an average of over 24,000,000 pollen grains, and that large panicles may occasionally produce over 100,000,000 pollen grains in the hermaphrodite flowers. Pollen deteriorated rapidly, and no seed was set when pollen was used 5 hours or more after it was collected from dehiscing anthers.

Stigmas were receptive at least 48 hours before the flowers bloomed and from 8 to 16 days after blooming.

A cytological examination of ovules collected at 2-hour intervals after pollination showed by the divided or undivided condition of egg or endosperm nuclei that fertilization takes place between 6 and 12 hours after pollination.

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STUDIES ON THE FERMENTATION OF TOBACCO[1]

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INTRODUCTION

After curing, cigar-leaf types of tobacco in particular are allowed to undergo one or more fairly definite periods of fermentation or "sweating." This process is characterized chiefly by an exchange of gases, the generation of heat, and a modification of the flavor and aroma of the leaf. The aging process in tobacco is not in all cases clearly distinguishable from fermentation, except that the rate of activity in the latter is more rapid and results in an appreciable liberation and accumulation of heat and gases. Although the subject of fermentation has received considerable attention in the past no satisfactory technic for measuring improvement in quality has been devised, and estimates of the progress of fermentation are dependent largely upon the opinion of those experienced in judging tobaccos.

As there is little exact knowledge concerning the nature of the process of fermentation, comprehensive investigations from several different angles will be necessary to establish the facts. The present investigations, in which the Dewar-flask method was used, are primarily concerned with the possible relationship between micro-organisms and the changes involved in the fermentation of cigar-leaf types of tobacco.

EARLIER STUDIES

The chemical changes occurring in tobacco during fermentation have been given particular attention by several investigators, but need not be reviewed here. It should be recalled, however, that tobacco fermentation is generally believed to be an oxidation process, and the close relation of air to the results secured has been generally recognized. Analyses show significant decreases in nitrogen compounds, including nicotine, and other organic substances, accounting for a loss of solid matter sometimes exceeding 5 percent, during fermentation. The total loss of weight, including that of free water, may be considerably higher during the process. On the other hand there is a marked liberation of ammonia and carbon dioxide gases as a consequence of this activity. The improvement of the aroma, flavor, burn, and other qualities is to be regarded as of major importance even though not chemically determinable. Separating the essential and desirable changes from incidental changes constitutes one of the chief difficulties of the chemical aspects of the problem.

The previous investigations which are of most interest in relation to the results secured in the present investigations are those dealing with the possible relationship of enzymes and micro-organisms to the fermentation process. There have been certain claims (*17*)[2] and

[1] Received for publication Mar. 12, 1934; issued August 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 159.

there is some evidence for the contention that, given the proper conditions, fermentative changes may occur in the absence of any enzymic or microbial activity, but this theory has not received much support. The problem seems rather to involve the relative importance of the enzymic and the microbial activities during normal fermentation, even though these may be regarded only as agencies speeding up the rate of oxidation and other chemical changes.

As early as 1858 an analogy between tobacco fermentation and alcoholic fermentation was implied by Koller (*10*), who added yeast with the purpose of increasing the rate of tobacco fermentation. Later, tobacco fermentation was compared with that of silage (*4*), "brown" hay (*1*), manures (*19*), etc. The bacterial theory of tobacco fermentation was most definitely brought forward in 1891 by Suchsland (*21*), who isolated bacteria from sweating tobacco, prepared pure cultures, and inoculated these back into tobacco. His claim that the flavor and odor of a specific type of tobacco could be developed in another type through the use of bacterial cultures has not, however, been substantiated. Miciol (*15*), Dávalos (*3*), Vernhout (*22*), Behrens (*1*), Koning (*11*), Jörgensen (*9*), and others soon afterward isolated organisms from tobacco and in general supported Suchsland's hypothesis. These workers showed that a variety of organisms were present, in what were considered large numbers, i.e., as high as 112,500 bacteria and 12,500 fungus spores on 100 cm^2 of freshly fermented leaf.

Suchsland's bacterial theory soon fell into disrepute under a vigorous though not convincing attack by Loew (*12*) in 1899. Loew not only claimed that bacteria were not present in sufficient numbers to influence fermentation, but that sufficient moisture was not normally present for their development, and that even if sufficient moisture were present, bacteria do not find tobacco a congenial medium for growth.

An even stronger argument against the bacterial hypothesis, however, was the enzymic theory of tobacco fermentation developed by Loew and treated in further detail in two succeeding papers (*13*, *14*). This theory ascribes tobacco fermentation to oxidizing enzymes normally present in all living material, as oxidase, peroxidase, and catalase, the latter being present in dried, cured, and fermented tobacco, even after several years. The chief role in fermentation was first ascribed to peroxidase, an enzyme readily identified by its reaction with tincture of guaiacum in the presence of hydrogen peroxide. Loew's theory has since been supported mainly by Boeckhout and Ott de Vries (*2*) and Jensen (*5*), but very little new evidence to support or controvert the enzyme theory has appeared in the literature. Jensen (*6*) in 1915 used the Dewar-flask method of study and concluded that fermentation of leaf tobacco cannot be inhibited through the addition of chemicals detrimental to micro-organisms. The data presented are not clear on this point, however, and the variability of the temperatures in the incubator used was such as to render the data of doubtful value. On the other hand, Jensen suggests the existence of two types of fermentation, namely, one which proceeds at a moisture content of 20 percent or below and another which requires a higher moisture content. Recent investigations in Russia, discussed in considerable detail by Smirnov (*20*), evidently are in general agreement with Jensen's conclusions.

It is evident from the literature that the determination of the nature of tobacco fermentation is complicated by the problem of what constitutes true fermentation and by the variation in the practical requirements of different types of tobacco.

Studies of a related nature have been conducted by many investigators on the spontaneous generation of heat in hay, straw, silage, manures, etc. The purely chemical, enzymic, and microbial explanations have all had stanch supporters, but it is interesting to note that recent investigations support the microbial theory (*16, 18*), at least under temperature and moisture conditions under which organisms will multiply, and even discount the cooperation of enzymes (*16*).

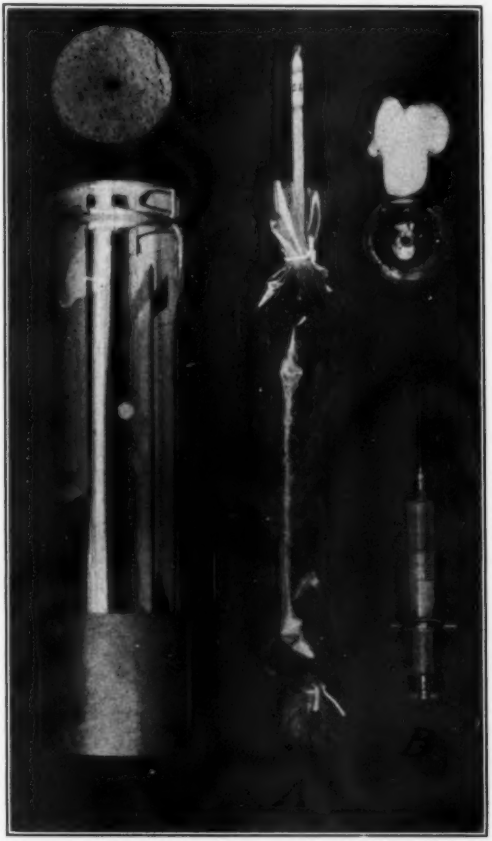

FIGURE 1.—A simple method for preparing tobacco samples for fermentation studies with pure cultures. The sterilized tobacco in the cellophane roll (*A*) may be inoculated at any desired points by inserting the syringe (*B*) through the cellophane before the roll is placed in the wide-mouth Dewar flask (*C*).

MATERIALS AND METHODS

At first the present studies were conducted with the ordinary narrow-mouth 1-quart thermos bottles. Later, wide-mouth flasks were secured which were easier to fill and permitted of handling the tobacco under fairly satisfactory aseptic and pure-culture conditions when desired. This was accomplished by first placing the tobacco in moisture-proof cellophane containers, sterilizing it with heat, and inoculating it with water suspensions of cultures of organisms by means of a Luer syringe inserted through the cellophane at one or more points. The cellophane containers were at first made the desired shape and size before being filled with tobacco; inoculations were then made by injections at a large number of points (fig. 1).

However, more even distribution of inoculum could be secured by preparing larger sealed cellophane bags in which the tobacco was held

loosely and into which the inoculum was injected with a syringe and mixed with the tobacco by turning and agitating it. The tobacco was then pressed into one end of the bag, which was rolled into form to fit the wide-mouth Dewar flask (fig. 2).

It was found that the tobacco could be adequately sterilized in these containers without apparent physical injury to the leaf or any appreciable change in moisture content by placing the roll or bag in a sealed copper container which was placed in an ordinary steamer. Forty-five minutes of steaming, during which the tobacco reached a temperature of 80° C., was sufficient to prevent subsequent thermogenesis, and plating out showed that no organisms were present. In practice,

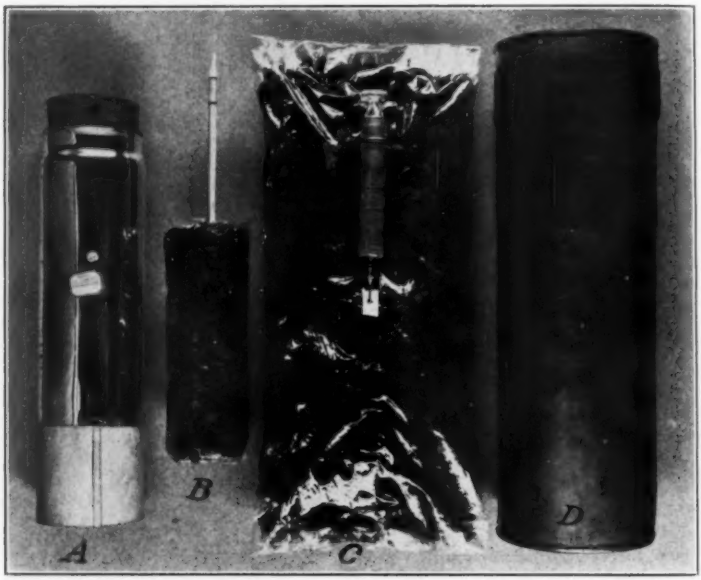

FIGURE 2.—The cellophane-bag (*A*, *B*, *C*) method permits of a uniform application of inoculum under aseptic conditions; the tobacco in the cellophane bag is sterilized in a copper, rubber-tube-sealed container (*D*), without undergoing any change in percentage of moisture.

however, the steaming was allowed to continue for 1 hour, during which time the tobacco reached a temperature of at least 85°. At these temperatures peroxidase was destroyed. In other tests peroxidase was found to be destroyed in water extract if heated for 10 minutes at 83°, and Loew (*12*) reports that the enzyme is destroyed at 87° in 3 minutes.

A limited amount of aeration was made available to the tobacco by various means. In the case of the narrow-mouth bottles a pliable perforated lead tube extended to the bottom of the flask. In the case of the cellophane containers, aeration was only provided by puncturing the cellophane at several points with the syringe or a hot needle and by not sealing the mouth of the flasks tightly. No other provisions for aeration were made, but since, as a rule, the tobacco was only loosely packed and the experiments continued for only 10 days, it is safe to

say that the amount of oxygen available was equal to that in normal fermentation in large boxes or bulks. At least, preliminary trials with air aspirated through the bottles did not appreciably increase thermogenesis, whereas the replacement of the air with nitrogen, followed by sealing, greatly reduced the thermogenic power.

The tobacco used in the tests was largely of the local variety known as Havana No. 142, grown on the Wisconsin Experiment Station farm. This tobacco contained approximately 30 percent moisture, which is close to normal for Wisconsin tobacco in the bale soon after stripping. The leaves were first stemmed and cut into strips about one-fourth inch in width; this made the pack more uniform and easier to handle. One hundred and fifty grams (about 5½ ounces) of this

FIGURE 3.—One of the constant-temperature incubators and the thermos bottles used in the earlier experiments.

tobacco was usually used in the 1-quart thermos bottles. The moisture content was usually raised to 35 percent or more by atomizing the tobacco with the desired quantity of distilled water. In most instances the chemicals were applied with the water.

As soon as the bottles were filled they were placed in automatically regulated constant-temperature chambers. Most of the experiments were run in a large 30° C. incubator in which the temperature normally varied less than 1° (fig. 3). More significant results would no doubt have been secured in some cases by incubating at a somewhat lower temperature. Readings were taken at 24-hour intervals (i.e., at 9 a.m.), usually over a period of 10 days. The temperature increases shown in the tables were then based on the average of the incubator readings subtracted from the average of the flask readings over the 10-day period. The maximum increases, which usually occurred about the fifth day, were considerably higher than the average. In most cases the incubator temperature recorded was

that of a thermometer inserted in a corresponding empty thermos bottle.

After the completion of the test in the thermos bottles, rough estimations of ammonia and carbon dioxide were usually made by drawing air from the aeration tube over concentrated hydrochloric acid and through limewater. These estimations of gaseous products are to be largely regarded as confirmatory information as to the direction and degree of activity exhibited. Exact quantitative determinations, while desirable, did not appear to be justified in the present connection, and would probably not have influenced the conclusions reached. The tobacco was then withdrawn from the flask, and a random sample of approximately one-half gram, or 10 square inches of leaf was introduced into about 5 cc of sterile water, agitated, and allowed to stand for about 1 hour before being plated out in nutrient agar or potato agar. Notes were also made at this time on the color of the

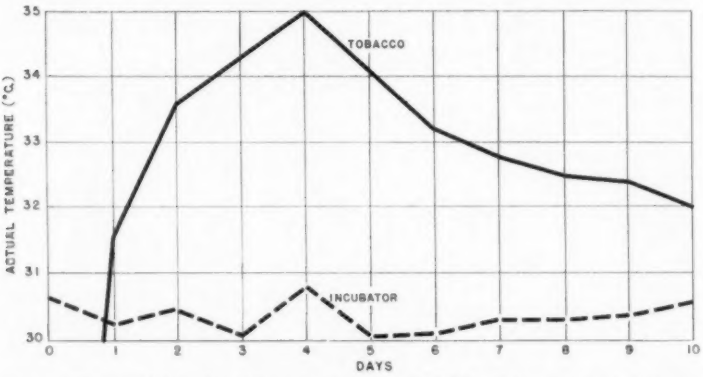

FIGURE 4.—Thermogenic behavior of tobacco in a thermos bottle held for 10 days at an incubator temperature of between 30° and 31° C.

water extract, and the odor, as well as on tests for peroxidase with tincture of guaiacum and hydrogen peroxide. The entire lot of tobacco was again weighed and then placed in an oven for determination of dry weight.

EXPERIMENTAL RESULTS

In the present investigation about 350 separate fermentation trials were made in Dewar flasks. The presentation of all the data secured seems unwarranted, and consequently only portions will be used to illustrate the conclusions drawn. The trials were rarely run in duplicate, preference being given to repeated successive trials, which were feasible since practically all environmental factors were under control and the data were based on average temperature readings. Control flasks, i.e., flasks containing untreated tobacco, were run, however, in connection with practically all trials.

The typical thermal behavior of such a control may be noted from the graph shown in figure 4. The tobacco being placed in the flask at room temperature and the flask then placed in an incubator averaging 30.5° C. (87° F.), the temperature rose very rapidly during the

first 24 hours as a consequence of both the heat from without and the thermogenesis from within, which in the presence of sufficient moisture usually gets under way the first day. The temperature normally continues on the upgrade in the flasks for 3 to 5 days, after which time it usually declines gradually. This decline is in part due to a reduction of the potential heat-producing constituents of the tobacco, since if this same lot of tobacco is removed and replaced in the flask with the original moisture content, the temperature curve is lowered and shortened. However, a gradual reduction in available oxygen and an accumulation of gases such as ammonia and carbon dioxide injurious to thermogenesis may also play a role in this decline.

EFFECT OF MOISTURE

The percentage of moisture in Wisconsin tobacco at the time it is baled on the farm averages approximately 30 percent,[3] the variation being generally between 25 and 40 percent. This tobacco may be fermented in several different ways. If the leaf is sorted, it is packed tightly in boxes or placed in bulks, where it goes through one or more sweats before being packed in boxes. If the leaf is stemming tobacco, the bales are stacked in large bulks, where they undergo fermentation. Following a period of aging, usually accompanied by natural drying, this leaf is moistened heavily before being stemmed, and the strips are again fermented in large bulks at a moisture content of about 40 percent. The results secured under the various methods of handling stemming tobacco may naturally be expected to be quite different, and the results in this type as a whole are apparently not comparable with those obtained in tobacco types stored at moisture contents of 20 percent or below, where little or no thermogenic activity occurs.

In large bulks or under high pressure, tobacco may evidently go through a normal fermentation process at a relatively low moisture content (22 to 28 percent). In thermos bottles with only 150 g of tobacco, loosely packed, no significant thermogenic activity was obtained at a moisture content of below 30 percent. As the moisture content is increased above 30 percent, the thermogenic power was found to increase proportionately (table 1). The rate of this increase was distinctly raised by increasing the pressure, i.e., placing 300 g of tobacco in the same volume (fig. 5). However, significant results by the thermos-bottle method are dependent upon having a moisture percentage of 35 to 40, which, though comparable with the amount of moisture present during the practical process of fermenting stemming tobacco, is not comparable with the usual percentage of moisture in sorted tobacco. It seems reasonable to assume, however, that within certain limits higher moisture contents in the thermos bottles for a time at least only serve to hasten activity of the same character as proceeds more slowly at lower moisture contents. In order that the generation of heat may be measurable in the thermos bottles, it must naturally accumulate at a more rapid rate than that at which it is lost through the insulating power of the flask. Furthermore, moisture is actually liberated during normal fermentation, as is suggested by the term "sweating", which no doubt renders the leaf and the environing air increasingly favorable for additional forms of activity. Taking

[3] The moisture percentages as given throughout this paper are computed on the basis of total weight.

all factors into consideration, limited generalizations with respect to the similarity of fermentation activity at ranges of from 25 to 40 percent are permissible. The situation in tobacco stored at moisture contents below approximately 20 percent may be expected to be of

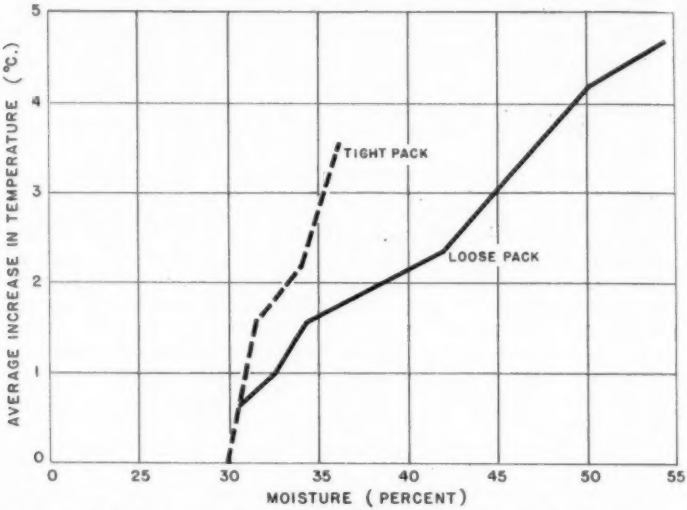

FIGURE 5.—Relation of percentage of moisture and tightness of packing to the thermogenic activity of tobacco in thermos bottles held at an average temperature of 30.2° C.

quite another nature, as are those in tobacco maintained at excessively high moisture contents.

TABLE 1.—*Relation of percentage of moisture in tobacco to its fermentation in Dewar flasks* [1]

Moisture (percent)	Average temperature			Ammonia	Carbon dioxide	Odor	Color of extract
	Incubator	Flask	Increase				
	° *C.*	° *C.*	° *C.*				
30.4	30.3	31.0	0.7	+	0	Raw	+
32.9	30.3	31.3	1.0	++	+	do	++
34.2	30.3	31.9	1.6	++	+	Mild	++
37.9	30.3	32.9	2.6	+++	++	do	+++
41.5	30.3	32.7	2.4	+++	+++	Good	+++
50.0	30.3	34.8	4.5	+++	+++	Strong	+++
54.3	30.3	35.0	4.7	+++	+++	do	+++

[1] In this and succeeding tables, plus signs indicate relative amounts, i.e., + small, ++ medium, +++ large.

EFFECT OF TEMPERATURE

Under favorable conditions cigar-leaf tobacco is ready for normal fermentation as soon as it is properly cured. In practice, some months may elapse between the completion of the curing and the beginning of fermentation. While this interim is partly a result of the time required for marketing, sorting, packing, and storage, fer-

mentation does not normally get under way until favorable natural or artificial temperatures are presented.

Little is definitely known about the most favorable temperature for fermentation, or regarding the lowest or highest temperatures at which it occurs. The solution of this problem presents many difficulties. An investigation of the problem by the Dewar-flask method suggests, however, many points of interest. The results of a series of trials are shown in table 2. The various temperature control chambers used could not be maintained regularly at a variation of less than 1° C.; consequently small temperature increases at the extremes were difficult to detect. Evidently, however, little or no activity occurred at temperatures below 10° C. (50° F.). At about 16° C. (61° F.) fairly evident thermogenesis began, reaching a maximum at 20° C. (68° F.) in this series. In other trials the indications were that the maximum of heat production lies closer to 25° C. (77° F.) (fig. 6). At any rate, spontaneous generation of heat begins

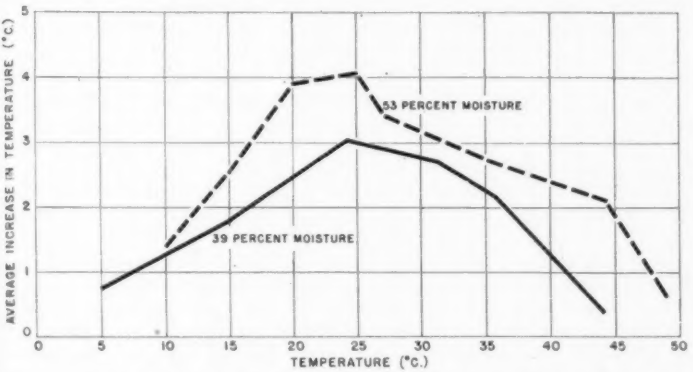

FIGURE 6.—Relation of temperature of incubation to the thermogenic activity of tobacco at two different moisture contents.

to drop off at 30° C. (86° F.) and is apparently entirely eliminated before a temperature of 50° C. (122° F.) is reached. Temperatures as high as 50° C. are rarely, if ever, reached in fermenting boxes of leaf tobacco, though in bulk fermentation considerably higher temperatures may be attained (*23*), whereas in stemming tobacco the bulk may be allowed to reach 60° C. (140° F.). For cigar wrapper and binder tobacco, however, it is now generally believed that temperatures above 50° C. are injurious to the quality of the leaf. Normally the bulk is therefore turned or the leaf transferred to boxes before this temperature is reached. The curve of the temperature rise in such a bulk is typically such as is shown in figure 7, in which it will be seen that following a rapid rise of temperature, the rate is diminished considerably beyond 43° C. (110° F.) and, in the fourth, fifth, and sixth rebulkings, drops off at increasingly lower temperatures, the primary reason perhaps being the lowered moisture content. In practice, bulks are now usually turned only once or twice before being boxed.

TABLE 2.—*Relation of temperature to the fermentation of tobacco in Dewar flasks*[1]

Moisture (percent)	Average temperature			Ammonia	Carbon dioxide	Odor	Color of extract	Peroxidase
	Incubator	Flask	Increase					
	° C.	° C.	° C.					
39.3	9.8	9.3	[2] −0.5	0	0	Raw	++	++
38.3	16.5	17.8	1.3	++	++	do	++	+++
39.1	20.0	23.7	3.7	++	+++	Good	+++	+++
37.8	25.4	28.0	2.6	+++	+++	Strong	+++	+++
37.0	27.0	28.4	1.4	+++	+++	do	+++	+++
37.3	36.6	38.4	1.8	+++	+++	do	+++	+++
37.0	44.1	44.9	.8	+	0	Mild	+++	+++
38.0	48.5	48.3	[2] −.2	0	0	do	+++	+

[1] See footnote, table 1. [2] Decrease.

Judging from the results obtained with the thermos bottles, fermentation apparently does not proceed much below 18° C. (65° F.)

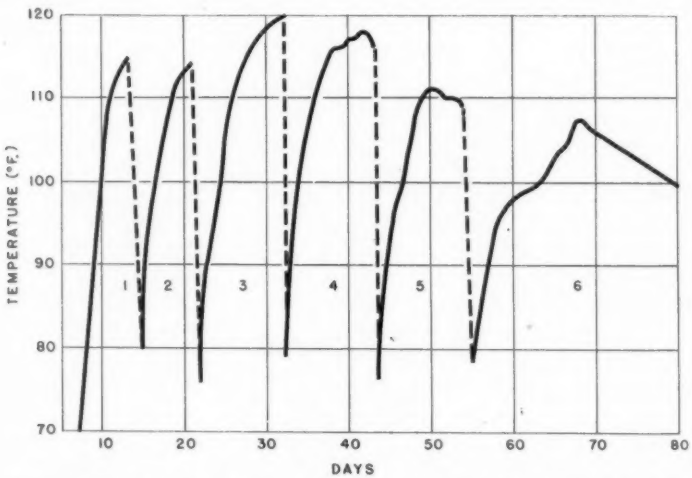

FIGURE 7.—Generation of heat in a large bulk of cigar-leaf tobacco following repeated rebulking.

or above 45° C. (113° F.). Several trials with tobacco "incubated" in a moist condition at temperatures between 45°–55° C. (113°–131° F.) for 20 days yielded little or no evidence of normal fermentative changes. On the other hand, there is some chemical evidence that heating tobacco directly may bring about changes resembling certain of those due to true fermentation, although evidently such heating cannot replace fermentation. Apparently, it is not the actual temperature reached in fermentation which is significant, but the actual increase in temperature above the surrounding atmosphere, since this increase represents the intensity of the process. Taking all facts into consideration, it will be seen that problems of considerable practical importance arise in this connection. Apparently fermenting rooms should preferably be maintained at approximately 22° C. (72° F.), and temperatures occurring in the fermenting leaf above

45° C. are less likely to be effective than are lower temperatures. A 30-degree increase in temperature between 75° and 105° F. should, for example, be considerably more effective than a 30-degree rise in temperature between 100° and 130° F. Hence, bulkings which result in excessive accumulation of heat may actually retard true fermentative changes.

EFFECT OF HEAT AND ANTISEPTICS

If tobacco is heated to a sufficiently high temperature in dry or moist heat, both the microbial flora and the enzymes present are destroyed. If such heated tobacco is placed in a thermos bottle in a moist condition, heat generation will still occur, though after some delay. This is true microbial thermogenesis, resulting from reinfestation with micro-organisms. If, however, the technic is modified so as to insure the introduction of heat-sterile tobacco into the container and the maintenance of aseptic conditions, thermogenesis is completely prevented, and the leaf is essentially preserved and unchanged (table 3). However, it is evidently not possible by the use of heat to destroy the microbial flora without destroying or injuring the plant enzymes or other constituents of the plant cells which may be concerned with normal fermentation, since the thermal inactivation points of most forms of living matter often too closely overlap.

It seemed quite conceivable, however, that certain chemicals, especially those of an antiseptic nature, might exert a selective action between microbial activity and the activity inherent in plant cells. Johnson and Murwin (*8*), for example, found in testing various disinfectants for tobacco seed, that while corrosive sublimate under certain conditions completely checked germination, silver nitrate under similar conditions was not injurious to the germination of the seed, a function evidently dependent upon enzymic activity. Both chemicals, however, were about equally destructive to micro-organisms. Chloroform, toluene, and acetone are usually conceded to be effective germicides, but with the additional property of not being particularly destructive to enzymes or harmful to their activity at low concentrations.

TABLE 3.—*Effect of heat applied to moist tobacco in air-tight copper containers on subsequent behavior in Dewar flasks when reinfestation with micro-organisms is prevented* [1]

Heat treatment of 150 g of tobacco	Average temperature increase	Ammonia	Carbon dioxide	Peroxidase	Bacteria	Fungi
	° C.					
None	4.3	+++	++	+++	+++	++
20 minutes, to 45° C	2.8	+++	++	+++	++	++
30 minutes, to 67 ° C	2.0	+	++	+	+	+
40 minutes, to 74° C	.2	+	0	0	+	+
45 minutes, to 80° C	[2] −.1	0	0	0	0	0
60 minutes, to 85° C	[2] −.2	0	0	0	0	0

[1] See footnote 1 table 1. [2] Decrease.

According to Loew (*14*), many salts and bases are not harmful to catalase activity, and some may be beneficial. Mercuric chloride however, was found to be distinctly harmful, as was formaldehyde in high concentrations (1 to 5). Chloroform was not found to be

destructive. While the data on this subject are not entirely clear in differentiating between destruction and inactivation, nevertheless the report by Jensen (*5*) that mercuric chloride, formol (formaldehyde) and chloroform do not prevent tobacco fermentation has been regarded by some as strong evidence in support of Loew's theory of enzymic fermentation, and good evidence against the microbial theory.

The results of the experiments here reported, in which the thermos-bottle method was used, have been quite contradictory to those of Jensen. Not only do mercuric chloride and chloroform effectively prevent thermogenesis, but acetone, toluene, beta-napthol, and other antiseptics at low concentrations also prevent thermogenic activity (table 4). The data secured with formaldehyde were less certain. Under the conditions of the experiments, both enzymes and microbes were apparently inactivated but not necessarily destroyed by many antiseptics. Chemicals other than antiseptics, such as ethylene chlorhydrin, acids, and bases, were tested with the purpose of discovering possible stimulatory action on thermogenic activity, but no conclusive evidence in this direction was secured.

TABLE 4.—*Effect of antiseptics and other chemicals on tobacco fermentation in Dewar flasks* [1]

Treatment of 150 g tobacco	Moisture	Average temperature			Ammonia	Carbon dioxide	Odor	Peroxidase	Staphylococci to the square inch [2]
		Incubator	Flask	Increase					
	Per cent	*° C.*	*° C.*	*° C.*					*Number*
None	37.0	30.3	33.7	3.4	+++	+++	Mild	+++	100,000
Acetone, 5 cc	37.0	30.3	30.7	.4	0	0	raw	+++	120
Toluene, 5 cc	37.0	30.3	30.7	.4	0	0	do	+++	0
Chloroform, 1 cc	37.8	30.3	30.5	.2	+	+	do	+++	
Formalin, 2 cc	51.1	30.0	31.7	1.7	+++	++	do	+++	500
Mercuric chloride, 1 g	37.1	30.5	30.8	.3	0	0	do	++	0
Silver nitrate, 1 g	37.1	30.2	31.7	1.5	+	+++	do	+++	0
Silver nitrate, 3 g	41.3	30.2	31.6	1.4	+	+++	do	+++	0
Ethylene chlorhydrin, 1 cc	43.8	27.7	30.3	2.6	+++	+++	Strong	+++	20,000
None	43.8	27.7	32.4	4.7	+++	+++	do	++	50,000
Potassium hydroxide, 2.5 g	38.5	30.5	33.4	2.9	+++	+++	do	++	100,000
Oxalic acid, 0.5 g	41.0	30.3	34.4	4.1	+++	++	do	+++	10,000

[1] See footnote 1, table 1. [2] Estimated.

The results secured with silver nitrate are of particular interest as indicative of a differential effect on microbial and enzymic activity. Under the conditions of the experiments, silver nitrate reduces thermogenesis to about one-half that of the untreated controls. The chemical, however, is quite as effective as the other antiseptics used in reducing microbial activity, as indicated by the freedom of the treated tobacco from surface fungus growth and from the results of plating out. Varying the quantity of silver nitrate from 0.25 to 4.0 g to 150 g of tobacco did not appear to affect the type of result secured. While the bacteria are evidently killed by the silver nitrate, many fungus spores may survive, raising some doubt as to the efficiency of the silver nitrate in checking fungus activity. The proof of their elimination by silver nitrate therefore must be sought by other means. This may evidently be accomplished by first destroying both the enzymes and the microbes in the tobacco by heat. Such tobacco will soon develop strong thermogenic activity as a consequence of micro-

bial development resulting from natural or artificial reinfestation. If, however, such heated tobacco is in addition treated with silver nitrate, no thermogenesis follows (table 5). It seems logical to conclude, therefore, that silver nitrate effectively checks microbial activity and that the thermogenesis occurring in unheated tobacco with silver nitrate is not due to microbial action, but is evidently due to some constituents (presumably enzymes) of the tobacco leaf itself, which are not harmfully affected by silver nitrate. The evidence suggests, therefore, that thermogenesis in tobacco may be brought about by enzymes alone, by enzymes in combination with micro-organisms, or by micro-organisms alone.

TABLE 5.—*Relation of silver nitrate to fermentative activity of tobacco in Dewar flasks* [1]

Treatment of tobacco, 150 g	Silver nitrate used	Artificial inoculation	Average temperature increase	Peroxidase	Bacteria	Fungi
	Grams		°C.			
Not sterilized	0	None	3.7	+++	+++	+
	1	do	1.5	+++	0	+
Sterilized	0	do	4.6	0	+	+++
	1	do	[2] −.1	0	0	0
	1	*Aspergillus flavus*	0	0	0	+
	1	*Aspergillus ochraceus*	.3	0	0	+
	1	*Aspergillus terreus*	0	0	0	+
	0	do	4.1	0	0	+++

[1] See footnote 1, table 1. [2] Decrease.

PRODUCTION OF AMMONIA

The most characteristic odor in rooms where large amounts of actively fermenting tobacco in bulks are being processed, is that of ammonia gas. According to Loew (*13*), this ammonia results from the destruction of the nitrogenous compounds by oxidizing enzymes and is not a product of putrefaction. The data secured in the present experiments repeatedly indicate the formation of large amounts of ammonia in the absence of oxidizing enzymes in the case of heated peroxidase-free tobacco. Ammonia production seems in all cases to be closely correlated with microbial activity and with thermogenesis. However, the determinations of ammonia here reported are only estimations made on the basis of the amount of fuming produced when air from the aeration tube was drawn over concentrated hydrochloric acid. More accurate determinations of correlation between enzymic activity and ammonia production must be made before it can be definitely concluded that peroxidase, catalase, or other enzymes are not also instrumental in the formation of ammonia in fermenting tobacco.

Ammonia production is only faintly discernible at low moisture percentages, where thermogenesis is not evident, but rises rapidly in direct proportion to the increase in moisture. At such low temperatures as 12° C. (54° F.) or below, ammonia production either is not determinable by the method used or is entirely absent even though slight thermogenesis has occurred. Its production then gradually rises up to about 25° C. (77° F.), and falls off again gradually to 44° C. (111° F.), with zero production again at 48° C. (118° F.) (table 2).

Mercuric chloride, acetone, and toluene may entirely prevent ammonia production, although measurable quantities developed in the presence of chloroform, beta-napthol, and Ceresan, in cases where thermogenesis was practically nil. Silver nitrate apparently checks ammonia production more effectively than it does thermogenesis. Other chemical agents used of a nonantiseptic nature had no depressing effect on the formation of ammonia.

PRODUCTION OF CARBON DIOXIDE

The formation of carbon dioxide is a result of oxidation and should therefore be closely correlated with the fermentation process. The data secured on this subject are again only estimations based upon drawing the atmosphere from the thermos bottles through limewater. As with the formation of ammonia, carbon dioxide formation is fairly closely correlated with thermogenesis, although it may often apparently be absent under conditions which permit some formation of ammonia. Its production is completely checked at low percentages of moisture and at the extremes of temperature. Most antiseptics usually completely prevent its formation, but silver nitrate usually reduced but did not check its production.

OTHER FERMENTATIVE CHANGES

The desired results of fermentation are those which bring about the best flavor and aroma in tobacco. While the term "aroma"is sometimes used in referring to leaf tobacco, it should preferably be reserved for cigars or manufactured tobacco and particularly for the smoking quality. The term "odor" will therefore generally be used for the changes which occur during fermentation. Chemically, there may be no particular relation between the odor and the aroma, although in practice a desirable odor is believed to bring about a desirable aroma.

The lack of any standard of measurement of odor, either qualitative or quantitative, makes it a difficult problem to attack. The ability of some persons to judge odor by the sense of smell is, to be sure, often remarkably developed, and, following much and varied experience, may be, for all practical purposes, sufficiently reliable for fine distinctions. In the present experiments it has been necessary to confine the estimations to rather wide differences in odor. The designation "strong" odor may be quite unsatisfactory, however, since it probably more correctly refers to the odor of ammonia, which may actually mask other odors of greater significance. The terms "raw" and "none", "mild" and "good" are more reliable. "Sweet" or "pleasant" odors were rather unusual types, secured at the higher temperatures, and may not have been of a true fermentative nature.

No fermentative odors are produced in the thermos bottles in the period of 10 days unless the moisture content is above about 32 percent, even though some heat production and some gases may be evident. At a 35-percent moisture content, however, a fair development of odor occurs at 30° C. (86° F.) in 10 days and apparently increases rapidly in direct proportion to the moisture present, up to a 45-percent moisture content or more, in which case a very strong but undesirable odor occurs in considerably less than 10 days. With a moisture content of 35 to 40 percent, no apparent fermentative odor develops below 18°, in 10 days. At temperatures beyond 20°, the

characteristic odor develops rapidly, reaching the maximum probably between 25° and 35°. Beyond 40°, the strength of the odor is strikingly decreased, and a sweet and more pleasant odor, sometimes perfumelike, results at temperatures up to 48°. At still higher temperatures it is doubtful whether any true fermentative odors are discernible.

Such antiseptics as were found to prevent active thermogenesis (mercuric chloride, chloroform, acetone, toluene, beta-napthol, etc.) also prevented the formation of any desirable or undesirable fermentative odors. Such chemicals as potassium hydroxide, oxalic acid, sodium carbonate, and ethyl chlorhydrin were without particular effect. Silver nitrate, while it permitted some thermogenic activity, checked the formation of fermentative odors quite effectively. Heating the tobacco to 80° C., or above, is in itself productive of some change in odor which may be confused with the odor of fermentation. However, it seemed apparent from a limited number of trials that the destruction of peroxidase by heat does not prevent the subsequent production of a fairly typical fermentation odor, when proper conditions for microbial activity are again brought about. Under such conditions there appears, however, to be more likelihood of certain fungi or other undesirable organisms developing excessively, with the resultant production of "wild" fermentative changes.

The color of a water extract of tobacco is of some value in comparing the relative degree to which the fermentative changes have progressed, although of little value in comparing different lots of tobacco with each other. As the fermentative activity increases, or the time is prolonged, the extract becomes increasingly darker in color; this condition is generally closely correlated with thermogenesis.

ENZYMIC RELATIONS

Although the presence of a number of different enzymes may be demonstrated in cured tobacco, it has not yet been conclusively shown that any particular enzyme, or these enzymes as a group, are responsible for fermentation in whole or in part. For the present, therefore, it may be satisfactory to select one of the easily determinable enzymes in cured tobacco as a general criterion of probable relation of enzymes to the experimental results secured. Peroxidase, as determined with tincture of guaiacum in the presence of hydrogen peroxide, was therefore chosen for this purpose. Unfortunately, this test does not distinguish between the presence of peroxidase in the active and in the inactive state; consequently, since it is normally present in tobacco, the significance of the present tests is largely limited to cases in which this enzyme is destroyed and hence eliminated as a factor in fermentation.

Peroxidase, as compared with living matter in general, is fairly resistant to unfavorable conditions. None of the antiseptics at the strengths used destroyed the peroxidase reaction with guaiacum. Since certain of these same antiseptics prevented thermogenesis, it must be assumed either that peroxidase is not connected with thermogenesis or that the enzyme was rendered inactive by the antiseptic. The latter assumption is quite logical.

In water extract from cured tobacco leaves peroxidase is destroyed at approximately 83° C., at a 10-minute exposure. When 150 g lots

of moist tobacco are placed in a dry-air oven for 24 hours, it normally required a temperature of 75° to 80° to destroy the peroxidase. In the moist condition peroxidase withstood a temperature of 49° for 10 days, though it was decidedly weakened by this treatment. Exposure to a temperature of 54° for 10 days in the moist condition destroyed peroxidase completely. It is therefore very unlikely that any thermogenesis resulting from peroxidase can occur above 50° C. (122° F.).

The behavior of enzymes and micro-organisms toward heat and antiseptics, as well as toward environmental conditions in general, is such that it becomes difficult to satisfactorily separate their activity in this manner in tobacco fermentation. Tobacco catalase, however, appears to be an exception. The optimum temperature for the activity of tobacco catalase as measured by the evolution of oxygen gas

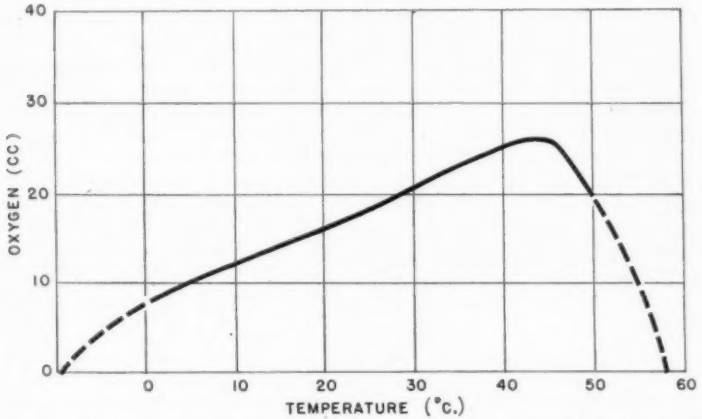

FIGURE 8.—Relation of temperature to the activity of catalase as measured by the evolution of oxygen from hydrogen peroxide.

with hydrogen peroxide was found to be close to 45° C., with the maximum at perhaps 58° and the minimum below 0° (fig. 8). According to determinations of others, the activity of catalase between 10° and 40° is more or less constant, and inactivation occurs at 55°. While the higher temperatures for catalase activity correlate roughly with all the fermentative changes and their decline at the upper limits, this correlation is no less evident for microbial activity.

On the other hand, there seems to be very little correlation between catalase activity and fermentative changes at the lower end of the temperature scale. If catalase is, for instance, approximately as active at 15° C. as at 25°, thermogenesis and other changes in tobacco should be quite comparable at these temperatures; whereas they are as a matter of fact strikingly different and more closely correlated with microbial behavior at such temperatures (fig. 9.).

Evidently it is necessary, however, to develop other forms of technic to distinguish enzymic from microbial activity in the case of active thermogenesis in tobacco. Theoretically, the destruction of all enzymes present by adequate heating, followed by the reintro-

duction of enzymes which may be concerned, together with the maintenance of aseptic conditions, should furnish conclusive proof of their connection. However, such evidence may, to be sure, lack definiteness except in the case of positive results. Using the cellophane-bag method for securing aseptic conditions, experiments of the above nature were performed in the following manner: One thousand grams of cured tobacco was extracted with 2,000 cc of water and yielded 1,300 cc of extract. This extract was filtered through a bacteria-proof porcelain filter, into approximately 50 cc lots. The filtered extract was stored for several days and remained sterile, as was shown by plating out from the separate lots. Unfiltered control extract soon became clouded with growth of organisms. These extracts yielded strong peroxidase reactions at dilutions as high as

FIGURE 9.—Relation of temperature of incubation of tobacco in Dewar flasks for the 10 days to the development of micro-organisms; *A*, 9.4° C.; *B*, 14.8°; *C*, 19.9°; *D*, 24.4°; *E*, 27.5°; *F*, 30.7°; *G*, 35.5°; H, 40.2°.

1 to 1,000. One-hundred-and-fifty-gram lots of sterilized, peroxidase-free tobacco in cellophane bags were then treated uniformly with these extracts in varying amounts ranging from 40 to 80 cc, the tobacco in these lots being thoroughly wetted by the larger amounts of extract applied. The bags were then rolled and introduced into the thermos bottles. Adequate controls were also prepared. In some cases contamination with organisms occurred as a consequence of providing for aeration, and the usual thermogenic activity resulted. However, in cases where contamination did not occur, no thermogenic activity developed, ammonia and CO_2 were hardly perceptible, and no changes in aroma were evident (table 6). Peroxidase and other enzymes or leaf constituents which are water-soluble were present, as was shown by tests at the end of the experiments, but the quantity of enzymes was evidently below normal. However, it is not unlikely that certain other conditions or activators necessary for enzymic activity were lacking; the results, being negative, are not regarded as conclusive. The method, however, offers possibilities of further development.

TABLE 6.—*Effect of applying varying amounts of filter-sterile extract containing peroxidase to sterile tobacco in Dewar flasks* [1]

Treatment of 150 g of tobacco	Filter-sterile tobacco extract added	Moisture	Average temperature increase	Ammonia	Carbon dioxide	Peroxidase	Fungi
	cc	*Percent*	° *C.*				
Sterilized	40	36.3	[2] −0.1	+	0	0	0
	60	43.2	.1	+	0	+	0
	80	46.0	[2] −.1	+	+	+	0
	[3] 60	43.8	4.1	+++	+++	+	++
Not sterilized	0	43.1	0	+	0	0	0
	0	35.0	3.3	+++	+++	+++	+

[1] See footnote 1, table 1. [2] Decrease. [3] Accidental contamination.

MICROBIAL RELATIONS

Many difficulties also lie in the way of furnishing conclusive evidence of any essential relation between micro-organisms and the fermentation of tobacco, and until the limits of fermentation are more clearly defined than at present, generalizations on this subject are premature. For those types of tobacco in which normal fermentation may be said to proceed at percentages of moisture that entirely preclude the development of micro-organisms, negative proof of microbial relationship should be quite simple. On the other hand, in the fermentation of tobacco in which the activity of micro-organisms may be readily demonstrated, it still remains to be shown that they play an essential rather than an incidental role. Such proof may be rendered difficult for several reasons, particularly the possibility that microbial activity under the conditions of the experiment may replace in whole or in part other agencies which may normally be responsible for fermentation.

The existence of a variety of micro-organisms in relatively high numbers in stored and fermenting tobacco has been repeatedly shown by different investigators. That certain species may find tobacco a favorable substratum for growth under certain conditions is amply demonstrated by the occurrence of such maladies as black rot, musts, and molds. The regularity and frequency with which still other species, which evidently cause no injury, occur suggest that these may exert some influence on fermenting and stored tobacco, although this influence may be admittedly harmful if allowed to progress too far.

In the present investigations over 350 platings have been made from the Dewar-flask-fermented lots, and from tobacco from various other sources, including widely separated tobacco-growing districts. The regularity of occurrence and the numbers of certain species of organisms were in some instances very striking. On the other hand, the relative infrequency of the occurrence of the known injurious forms, such as *Aspergillus niger* (7) and *Oospora nicotianae* in these plates suggests the limited degree of infestation necessary to produce subsequent extensive changes in tobacco under conditions favorable for the development of the particular species concerned. Two large, white-colony forms of bacteria of undetermined species were commonly present, but these were not regarded as significant for the double reason that no marked increase in numbers occurred during

fermentation and because they were evidently unable to induce thermogenic activity in tobacco when introduced in pure culture. On the basis of almost universal association with stored and fermenting cigar tobacco, a tiny coccus form seemed worthy of more consideration (fig. 10). This organism has regularly been secured on nutrient agar from fermenting tobacco from widely separated sources (table 7), often in numbers indicating 300,000 to 500,000 organisms to the square inch of leaf surface. Preliminary studies on this organism in

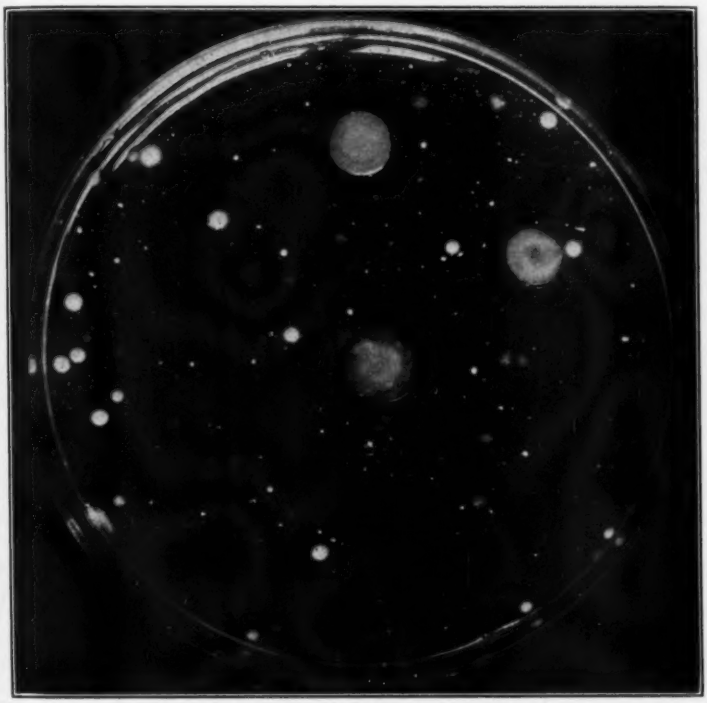

FIGURE 10.—Large numbers of a *Staphylococcus* species secured in dilution plates from fermenting tobacco, and the relative size of these colonies as compared with three colonies of large bacteria.

cooperation with the Wisconsin Department of Bacteriology indicate that it belongs to the *Staphylococcus* group. While the regularity of occurrence and the numerousness of this organism in fermenting tobacco suggest some relation to the fermentation process, it also failed to induce thermogenic activity in tobacco under the conditions of the experiments.

The fungi encountered in stored and fermenting tobacco are normally fairly common species in nature, though little is known about what significance varieties or strains of these fungi may have in the present connection. *Aspergillus flavus* Lk., *A. terreus* Thom, and *Penicillium brevicaule* Sacc. were commonly found in fermenting cigar-leaf tobacco and were used in connection with the present

studies. *A. ochraceus* Wilhelm, a form growing abundantly on a sample of flue-cured tobacco, was included in the studies, as was *A. niger* Tiegh., the causal organism of black rot of fermenting tobacco. These species were either determined or verified by Charles Thom, of the Bureau of Chemistry and Soils, United States Department of Agriculture, from cultures submitted. In contrast with the bacteria, these fungi were found to be very active thermogenically on tobacco and consequently should be given more consideration as possible factors in fermentation, whereas previous students of the microbial hypothesis considered the bacteria of major consequence.

TABLE 7.—*Fermentation results in Dewar flasks with tobacco from various cigar-tobacco growing districts* [1]

Source of tobacco and year grown	Variety	Moisture	Average temperature			Ammonia	Carbon dioxide	Odor	Estimated cocci to square inch	
			Incubator	Flask	Increase				In flasks	Control
		Percent	*° C.*	*° C.*	*° C.*				*Number*	*Number*
Ohio (1931)	Dutch	39.0	23.9	26.9	3.0	+++	+++	Strong	100,000	50,000
Do	Zimmer	38.3	23.9	26.4	2.5	+++	+++	do	150,000	80,000
Connecticut (1930)	Havana seed.	38.5	23.9	25.2	1.3	+	+	Mild	100,000	1,000
Connecticut (1931)	do	35.0	23.9	26.6	2.7	+++	+++	Good	100,000	200
Massachusetts (1931)	do	38.0	23.9	26.1	2.2	+++	+++	do	80,000	0
Canada (1929)	do	44.2	23.9	27.2	3.3	++	+++	do	100,000	3,000
Wisconsin southern (1931)	do	27.3	23.9	23.9	0	+	0	Raw	30	0
Wisconsin northern (1931)	do	33.0	23.9	26.7	2.8	++	+	Slight	100,000	30
Pennsylvania (1931)	do	41.2	23.9	29.0	5.1	+++	+++	Strong	150,000	40,000

[1] See footnote 1, table 1.

TABLE 8.—*Effect of pure cultures of various organisms on sterilized tobacco in Dewar flasks* [1]

Inoculum	Moisture	Average temperature increase	Ammonia	Carbon dioxide	Peroxidase	Color of extract
	Percent	*° C.*				
Aspergillus niger	37.0	4.2	++	+	0	++
Aspergillus ochraceus	36.1	3.6	+++	+	0	+
Aspergillus terreus	37.7	6.1	+++	++	0	+++
Aspergillus flavus	37.8	2.8	+++	+	0	+++
Penicillium brevicaule	40.0	2.7	+++	+++	0	+++
Penicillium baiiolum	37.5	.3	++	0	0	+
Oospora sp.	39.1	.6	++	0	0	+
Bacterium sp. 1	38.7	[2] −.2	0	0	0	+
Bacterium sp. 2	40.1	.1	0	0	0	+
Staphylococcus sp.	39.0	.1	0	0	0	+
Not inoculated; control	38.1	0	+	0	0	+
Not sterilized; control	39.8	5.1	+++	+++	+++	++

[1] See footnote 1, table 1. [2] Decrease.

By using the cellophane bags in the Dewar flasks, it was possible to first eliminate all possible agencies of fermentation by heating the tobacco for the required length of time. The above-mentioned organisms carried in pure culture could then be subsequently introduced for comparative studies. The bacteria, as has been noted, caused no appreciable thermogenic activity in tobacco, although the *Staphylococcus* form developed in large numbers. Neither did the

bacteria affect any other measurable changes in the leaf under the conditions of the tests. Certain fungi, however, not only usually induced a striking generation of heat, and a resultant production of ammonia and carbon dioxide, but developed odors and other characteristics fairly typical of fermenting tobacco (table 8). Other fungi evidently were capable of little or no influence of a fermentative nature. Detailed studies are required, however, before satisfactory conclusions can be drawn in regard to the comparative activity and actual influence of these and other fungi on the fermentation process under both experimental and commercial conditions.

DISCUSSION OF RESULTS

As understood in the present investigation, the fermentation of cigar-leaf tobacco is an oxidation process resulting in the formation of carbon dioxide and the generation of heat, together with a general improvement in the desired qualities of the tobacco, usually accompanied by the production of ammonia. The influence on quality may be of various kinds, but in general the process eventually removes a "green" or raw taste and odor and develops an aroma. The immediate result may often be the formation of such amounts of ammonia and other products as to render the leaf excessively strong, and the benefits of subsequent storage and aging are in part at least due to the gradual loss of such objectionable substances.

The oxidation process may no doubt be brought about in different ways or at least at markedly different rates, depending upon the environmental conditions. The results secured in the present studies have not eliminated any of the hypotheses held at present as to the nature of the causative agent in fermentation but have rather added some support to both the enzymic and microbial hypotheses. The latter hypothesis has been in danger of being totally discarded from consideration without all the evidence being taken into consideration. Loew's (*12*, *13*) conclusions, for instance, are particularly weak with respect to microbial behavior in tobacco, though his evidence for "oxidizing enzymation" is convincing. Evidently the chief basis for denying any essential relationship between fermentation and microbial activity is the low moisture content present in tobacco types which are "reordered" or redried before being placed in storage. Flue-cured tobacco, in particular, is much more susceptible to fungus development than is cigar tobacco, and unless stored in a condition relatively dry as compared with storage conditions for cigar-leaf tobacco, usually becomes overgrown with fungi to a damaging extent. However, neither the results nor benefits of fermentation as contrasted with those of aging are clearly defined in redried tobacco, and it is quite conceivable that a slow oxidation, entirely independent of microorganisms, may proceed in such tobaccos. On the other hand, millions of pounds of stemming tobacco and cigar tobacco are regularly stored or processed in such a manner as to render microbial development certain. It is not unlikely that this procedure has some essential relation to the desired results. Whether this procedure is to be regarded as of the nature of fermentation or special processing may be open to question.

While it has been claimed by Jensen (*6*) and by Smirnov (*20*), on the basis of trials with antiseptics, that fermentation may proceed

in the absence of microbial activity, the data presented are not convincing on this point. Evidently the selective influence of various antiseptics on enzymes and microbes under different circumstances needs more intensive study. Silver nitrate appears to possess this differential property more completely than any of the other antiseptics used, and, on the basis of the results secured with this chemical, it seems evident that fermentation may proceed in the absence of microbial activity. On the other hand, fermentative changes of an apparently normal character may evidently be brought about by various fungi in tobacco heated sufficiently to destroy all other forms of activity. Bacteria, which have previously been generally believed to be concerned in this process, were not found to be capable of developing thermogenic activity.

SUMMARY

Microbial and enzymic factors which may be concerned with the fermentation of cigar-leaf tobacco were investigated by the Dewar-flask method. Modifications of technic were used which permitted of operations under aseptic conditions and inoculations with pure cultures of micro-organisms.

With 150 g of tobacco, average temperature increases in Dewar flasks over a 10-day period ran as high as 5.6° C. In general, the generation of heat (thermogenesis) was directly proportional to the percentage of moisture present, a minimum of about 30 percent being necessary to secure measurable activity under the conditions of the experiments.

The highest thermogenic increases occurred at incubator temperatures of about 20° to 25° C., very little if any activity developing at temperatures below 10° C. (50° F.) or above 45° C. (113° F.). This experimental maximum temperature is considerably lower than the temperature often allowed in practice by the bulk-fermentation method, and suggests the possibility of obtaining better results at more moderate temperatures.

Chloroform, mercuric chloride, acetone, toluene, or beta-napthol may almost completely check thermogenesis. These antiseptics check microbial activity, and although they do not destroy peroxidase and other enzymes, they apparently cause inactivation. Silver nitrate, on the other hand, reduces thermogenesis under similar conditions to only about one-half that which normally obtains. It is believed that silver nitrate prevents microbial activity without being harmful to the action of enzymes under the conditions of the experiments.

Heating the tobacco to a sufficiently high temperature will completely check thermogenesis or any other expression fermentation, provided aseptic conditions are maintained. Treating such tobacco aseptically with porcelain-filtered extract from unheated tobacco containing peroxidase and other enzymes failed to induce thermogenesis.

Three species of bacteria commonly occurring on tobacco failed to induce thermogenic activity in heated tobacco, but several fungi isolated from tobacco were very efficient in this respect, yielding results thermogenically and otherwise comparable to normal fermentation.

The evidence appears to justify the conclusion that micro-organisms, especially fungi, may play a role in the fermentation or in the

processing of certain types of cigar-leaf tobacco, although such organisms may not necessarily be essential to fermentation.

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THE NUTRITIVE VALUE OF GREEN IMMATURE SOYBEANS[1]

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INTRODUCTION

Much information is available regarding the nutritive value of dry soybeans and products made from them, whereas the green immature soybeans have been little studied. When it was learned that orientals, especially Japanese, in Hawaii as well as in the Orient, use the green soybeans, experiments were planned to obtain information regarding the composition and vitamin content of the local product.

Green immature soybeans look much like young, tender lima beans, but they have a richer, more distinctive, and more delicious flavor. It is usually desirable to cook them in the pods, boiling them in salted water from 15 to 25 minutes. The beans then slip easily from the pods and can be used in salads or in soups and as a vegetable. The orientals often eat them directly from the pods, especially between meals. Children sometimes carry about a small paper bag of the cooked beans, break open the pods, and then lick the beans out with their tongues—a practice to be recommended from the sanitary as well as from the nutritive standpoint.

Chung and Ripperton (*2*)[2] have reported determinations of calcium, phosphorus, iron, and other nutritive constituents in raw green soybeans raised in Hawaii. Saiki and coworkers (*8*) have reported similar analyses from Japan. Piper and Morse (*7*) have reported one analysis, and Muramatsu (*6*) has studied the comparative starch content of green and dry soybeans. No reports of vitamin studies of raw or cooked green soybeans have been found in the literature.[3]

METHODS AND EXPERIMENTAL DATA

COMPOSITION OF GREEN SOYBEANS

The nutritive constituents and mineral elements of the raw and of the cooked green, immature soybeans were determined by the methods of the Association of Official Agricultural Chemists (*1*) or by slight modifications of these procedures. Iron was determined by the method recommended by Elvehjem and Hart (*3*).

Through the courtesy of C. P. Wilsie, agronomist of the Hawaii Agricultural Experiment Station, one of the varieties of beans used for the analyses and feeding tests was grown on the university farm. The seeds of these soybeans (Seaweed, F.P.I. no. 80483) were obtained from W. J. Morse, of the United States Department of Agriculture, who had obtained them from Japan and who stated that they were used only in the green state. When mature, the seeds are large and flat, with a testa pattern of a black saddle on a yellow base. In

[1] Received for publication Mar. 21, 1934, issued August 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 167.
[3] Brief reference is made to such studies by Miller (*5, p. 2*).

the fresh green state in which they were analyzed they were light green with a purplish-red saddle that changed to bluish-gray on cooking.

Beans of unknown variety bought on the open market appeared bright green after cooking and were not so flat in shape as the Seaweed variety.

For the analyses, the fresh green soybeans were removed from the pods and dried in large evaporating dishes in an enamel-lined oven at a temperature below 80° C. The cooked green soybeans were prepared by boiling in salted water for 20 to 25 minutes, after which the beans easily slipped from the pods and were dried in the same manner as the fresh soybeans. For the iron analyses, the soybeans were ashed wet to avoid contamination.

Table 1 summarizes the analyses of one variety (F.P.I. no. 80483) in the uncooked and in the cooked states, and of a local unknown variety in the cooked state only.

TABLE 1.—*Composition of fresh green soybeans*

Treatment of green soybeans	Moisture	Protein	Ether extract	Crude fiber	Ash	Carbohydrates (by difference)	Calcium	Phosphorus	Iron
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Cooked [1]	64.2	15.0	2.7	1.6	2.9	13.6	0.100	0.257	0.00213
Do. [2]	63.5	13.8	4.2	1.5	3.1	13.9	.098	.286	.00344
Raw [3]	69.1	12.5	5.1	1.5	1.8	10.0	.063	.239	.00283

[1] Variety unknown; purchased on open market: 2 samples.
[2] Variety (F.P.I. no. 80483); grown on university farm; 2 samples.
[3] Variety (F.P.I. no. 80483); grown on university farm; 1 sample.

The analyses show that the protein content of green soybeans is from 12.5 to 15 percent, an unusually large percentage for a fresh green vegetable.

Muramatsu (*6*) reports that the dry soybeans contain little or no starch, but that the unripened, green soybeans contain from 2.6 to 4.76 parts per 100 parts of dry material. Investigations of others cited by Muramatsu show the nitrogen-free extract of dry soybeans to be made up of sugars, dextrin, pentosans, galactans, cellulose, waxes, and coloring matter. Consequently, in table 1 the figures given for carbohydrates by difference, constituting approximately 14 percent of the cooked soybeans, do not represent all utilizable carbohydrate.

The figures for ether extract show from 3 to 4 percent of fat in the cooked green soybeans as compared with 12 to 18 percent in four varieties of dried soybeans reported by Wu (*11*).

The crude fiber of the green, immature soybeans is 1.5 percent, as compared with 3.4 to 6.8 percent for four varieties of dry soybeans reported by Wu (*11*). This low crude fiber content should permit of a higher percentage utilization of the organic nutrients and of the minerals in the green soybeans than in dry legumes.

The energy value of cooked green soybeans is relatively high, as it takes only 70 g or one-half of a cupful to yield 100 calories.

The calcium content of 0.1 percent compares favorably with that of milk and is much higher than in most vegetables. Moreover,

the phosphorus content of 0.286 percent is much higher than that of other vegetables or milk. The iron content of green soybeans exceeds that of most other common vegetables reported by Sherman (*9*).

Analyses made by the authors confirm those previously reported for the fresh raw soybeans and indicate that cooked green soybeans are a vegetable of great nutritive value in the oriental diet. People of other countries might well add them to their diet.

VITAMIN METHODS AND DETERMINATIONS

The methods used for the quantitative determination of the vitamins were essentially those recommended by Sherman (*9*). The rats were standard animals raised by the Hawaii Station. All the usual precautions recommended for carrying out quantitative vitamin tests were followed.

Both of the varieties previously referred to were used for the feeding tests, but no difference could be detected in their vitamin content, either when fed during the same or different experiments. Twice a week a fresh supply of beans was cooked in the pods in salted water for 25 minutes. The beans were removed from the pods and stored in a tight-fitting glass jar in an electric refrigerator until used.

The supplements were weighed on a chainomatic balance and were fed daily, except the 0.25-g supplements for the vitamin A experiments which were fed as 0.5 g three times a week.

The term "daily feedings" is here used to mean feedings given every day except Sunday.

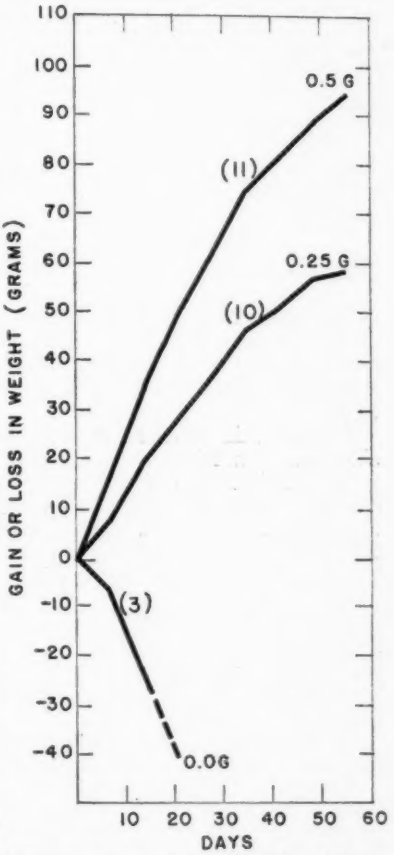

FIGURE 1.—Average gain in weight of groups of young rats fed various amounts of cooked immature soybeans as the sole source of vitamin A. The broken line indicates the occurrence of the first death in the control group. The numbers in parentheses show the number of rats used for each test.

VITAMIN A

For the vitamin A experiments, young rats were weaned and placed on the experimental diet at the age of 3 weeks, when they weighed an average of 40 g.

Table 2 and figure 1 summarize the results of feeding cooked green soybeans as the sole source of vitamin A to 21 standard white rats. One rat fed daily 0.5 g of green soybeans and two fed daily 0.25 g succumbed several weeks after the supplements were started. Their records are not included in the results.

TABLE 2.—*Results of feeding various amounts of cooked green soybeans to rats as the sole source of vitamins A, B, and G*

Vitamin studied and source	Rats	Weight of supplement fed daily	Average weights			Average gain (+) or loss (−) in 8 weeks
			Initial	When supplement started	Final	
Vitamin A:	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Negative control	3	0.00	37	[1] 110	81	----
Green soybeans, cooked	10	.25	40	119	178	+59
Do.	11	.50	41	121	216	+95
Vitamin B:						
Negative control	2	0.00	52	[1] 73	50	----
Green soybeans, cooked	8	1.00	57	80	102	+22
Do.	7	1.30	57	79	110	+31
Vitamin G:						
Negative control	3	0.00	46	[1] 55	54	−1
Green soybeans, cooked	2	1.00	47	52	65	+13
Do.	12	1:50	48	58	101	+43
Do.	7	2.00	46	54	102	+48
Do.	2	4.00	46	54	152	+98

[1] Weight at the end of the depletion period.

The growth curves indicate that green cooked soybeans are a very good source of vitamin A. Ten rats gained an average of 59 g in 8 weeks when fed daily 0.25 g of cooked green soybeans, and 11 rats gained an average of 95 g in 8 weeks when fed daily supplements of 0.5 g.

Despite this excellent growth response many of the rats showed abnormal conditions at autopsy. Three rats fed the 0.5-g supplements had bladders with thickened and hemorrhagic walls containing calculi. Three showed kidneys with enlarged sinuses. Six rats fed the 0.25-g supplements had abnormal bladders with hemorrhagic and thickened walls. Three showed kidneys with enlarged sinuses. Three had slightly swollen eyelids.

All of the male rats on the 2 supplements showed poor sexual development; the testes were practically always small, and microscopic examination showed few if any mature sperms in the cauda epididymis. On the other hand, the females were better developed sexually and their ovaries appeared to be normal with ripening or large ripe follicles.

Twelve animals, 6 in each group, tended to drag their hind legs and had as a result a very unsteady gait.

No attempt was made to feed supplements smaller than 0.25 g, since it had been observed that rats gaining much less than 60 g in the 8-week period suffered such high mortality from kidney and bladder infections, and occasionally from lung infection, that it was useless to try to obtain gains of 25 to 30 g in 8 weeks.

VITAMIN B

Young rats weaned when 25 and 26 days old averaged 57 g in weight when placed on the vitamin-B-free diet.

The results of feeding 15 standard rats cooked green soybeans as the sole source of vitamin B are summarized in table 2 and figure 2.

The growth curves show that cooked green soybeans, like most legumes, are a good source of vitamin B. Eight rats fed daily supplements of 1 g of cooked green soybeans gained an average of 22 g in 8 weeks, and 7 rats fed 1.3-g supplements gained an average of 31 g in 8 weeks.

Nothing noteworthy was observed at autopsy except that the females showed less sexual development than the males. The ovaries were small and undeveloped with no ripening follicles, whereas most of the males had moderately well developed testes with microscopic evidence of motile sperms in the cauda epididymis.

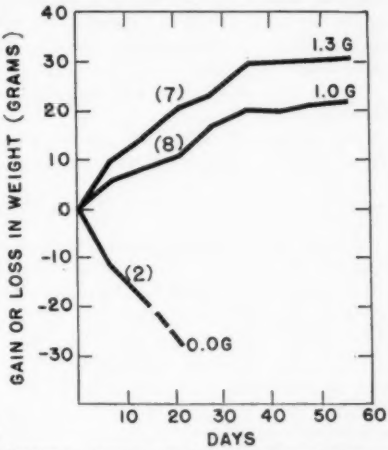

FIGURE 2.—Average gain in weight of groups of young rats fed various amounts of cooked immature soybeans as the sole source of vitamin B (B_1). The broken line indicates the occurrence of the first death in the control group. The numbers in parentheses show the number of rats used for each test.

VITAMIN G (B_2)

Young rats weaned when 23 and 26 days old averaged 46 and 48 g in weight when placed on the vitamin-G-free diet.

The results of feeding 21 rats cooked green soybeans as the sole source of vitamin G are summarized in table 2 and figure 3.

For these experiments, vitamin B was supplied the rats by feeding daily 4 drops (0.12 to 0.13 g) of tikitiki extract obtained from the Bureau of Science, Manila, P.I. The fact that the three controls maintained a practically constant weight during the 8-week period following the depletion of vitamin G stores, is evidence that this quantity of tikitiki extract contributes little or no vitamin G to the diet.

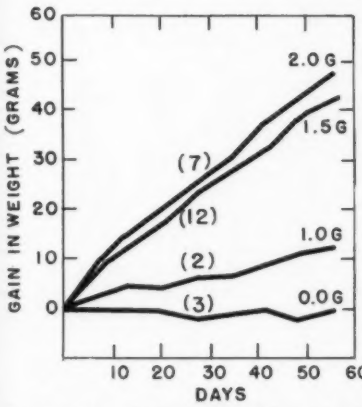

FIGURE 3.—Average gain in weight of groups of young rats fed various amounts of cooked immature soybeans as the sole source of vitamin G (B_2). The numbers in parentheses show the number of rats used for each test.

Judging from the growth curves, cooked green soybeans may be considered a good source of vitamin G. Seven rats fed daily 2 g of cooked soybeans gained an average of 48 g in 8 weeks and 12 rats fed 1.5 g daily gained an average of 43 g in 8 weeks. The gains were very uniform and there was no flattening of the growth curves after the fifth or sixth week of the test period.

Two rats, whose records are included in table 2 but not in the figure, were fed 4 g of cooked soybeans daily and gained 96 and 99 g during the 8-week test period.

At autopsy all the rats had abnormally short, soft fur, which in some cases was thin around the head and shoulders. All except one showed varying amounts of blood on nose, paws, and ears, which came from the hair follicles at the base of the whiskers. Only two of the rats showed small lesions. No other symptoms of G avitaminosis were observed.

As in the vitamin B experiments, the females showed poorer sexual development than the males. The ovaries were small without ripe or ripening follicles, but most of the males showed moderately well developed testes and motile sperms.

Vitamin C

Fourteen standard guinea pigs raised in the nutrition laboratory were used to test the vitamin C potency of cooked green soybeans. The guinea pigs were fed Sherman's (*9*) scorbutic basal diet plus fresh alfalfa plus the supplements until they ate the soybeans readily. The alfalfa was then discontinued and the soybeans fed to the guinea pigs for the periods indicated below. At the end of the experiment the histological examination of the incisor teeth recommended by Höjer (*4*) was employed. The results of the experiments are summarized in table 3.

Table 3.—*Results of feeding various amounts of cooked green soybeans to guinea pigs as the sole source of vitamin C*

Guinea pig no.	Weight of supplement fed daily	Period during which supplement was fed	Weights of animals—		Net gain or loss	Gross scurvy at autopsy [1]	Histological examination of teeth, Höjer's rating [2]
			When supplement started	At end of experiment			
	Grams	*Days*	*Grams*	*Grams*	*Grams*		
232–F	0		366	388	82	+++	0.2
234–F	10	21	296	317	21	tr.	.4
236–F		21	307	256	−51	+	.4
238–F		21	296	330	34	tr.	.4
269–M		21	355	436	81	−	.7
267–M		21	302	375	73	−	.8
289–M		21	321	406	85	−	.9
293–M [3]	12	21	301	318	17	+	.4
264–F		21	324	364	40	−	.7
295–M		61	335	599	264	−	.5
291–M		21	320	418	98	−	.5
258–F [3]	15	21	298	296	−2	++	.4
254–F		60	304	462	158	−	.7
256–F [3]	20	21	300	313	13	+	.4
252–F		60	315	442	127	−	1.0

[1] Sherman, H. C., and Smith, S. L. (*10, p. 173*).
[2] Höjer, A. (*4*).
[3] Animal did not eat supplement satisfactorily.

The animals were somewhat erratic in their response to the feeding of cooked green soybeans as a source of vitamin C; some in each group showed evidence of gross scurvy at autopsy. Guinea pig no. 293–M, fed 12 g, and no. 258–F, fed 15 g, did not eat all of the daily supplement during the last week of the experiment. Guinea pig no 256–F, fed 20 g, frequently failed to eat the daily supplement immediately after it was given but often left a part of it for 5 to 6 hours or longer. This probably accounts for the fact that these three guinea pigs showed

evidence of gross scurvy, whereas the other animals fed similar daily supplements were protected.

The results of the feeding tests indicate that cooked green soybeans are a poor source of vitamin C, as daily 12-g supplements are required to protect the animals from gross scurvy. Apparently 20 g or more are necessary to protect the teeth from histological changes, but it is difficult to induce guinea pigs to eat this quantity.

SUMMARY

Two varieties of cooked green immature soybeans were analyzed for organic nutrients and for calcium, phosphorus, and iron.

As compared with most vegetables, they had unusually large amounts of protein, fat, calcium, phosphorus, and iron.

Cooked immature soybeans proved to be a very good source of vitamins A, B, and G and a poor source of vitamin C.

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THE NUTRITIVE PROPERTIES OF PROTEIN, VITAMINS B AND G, AND THE GERM IN RYE[1]

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INTRODUCTION

It has been the experience of numerous investigators that rye, *Secale cereale* L., has less value than corn, wheat, or barley as a feed for swine. Practically all of the investigators who have worked with swine have been interested either in comparing the feeding value of rye with that of one or more of the other cereals or in finding satisfactory methods of feeding the grain and have not endeavored to determine the cause of its low feeding value.

Judging from its proximate analysis, rye should possess a higher nutritive value than the other cereals. According to the data compiled by Henry and Morrison (*7, pp. 709–710, 728–729*)[2] rye contains more crude protein than barley or corn, more digestible protein than any of the cereals, and somewhat less fiber than the other grains.

REVIEW OF LITERATURE

Many investigators (*4, p. 7; 9; 11; 24*)[3][4][5] have found rye to be very unpalatable to swine. It is impossible to determine to what extent this unpalatability can be attributed to the rye itself, for this grain is usually contaminated with the fungus, *Claviceps purpurea* (Fr.) Tul., which has been shown by Sackville and Sinclair[5] to be very distasteful to swine. Digestive disturbances from the feeding of rye to swine, as evidenced by diarrhea, have been reported by Ferrin and McCarty[6] and by Wilson and Wright (*24*). That the feeding value of the rye could not be enhanced by providing a source of vitamin A or D was demonstrated by Ferrin and McCarty[6] by feeding one-half ounce of cod-liver oil daily per pig. These investigators[7] obtained satisfactory results for the first time by the use of liver meal, a special product made from livers, as a supplement to rye, although this product in an earlier trial[8] had not improved a rye ration.

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[1] Received for publication Jan. 31, 1934; issued August 1934. This article was presented by the senior author to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy August 1933. Paper no. 1245, Journal Series, Minnesota Agricultural Experiment Station.

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[8] FERRIN, E. F., and MCCARTY, M. A. See footnote 3, fourth citation.

Although the majority of the investigators have found rye to be much less desirable as a feed for swine than other grains, some have reported quite satisfactory results; thus Faville (*3, pp. 4–5*), Edwards and Brown (*2*), and Snyder (*21, p. 44*) have found rye to be practically equal to other grains. Likewise Morgan and Arnett (*18*) have reported a series of rye-feeding experiments in which rye proved to be a satisfactory feed.

There are but few experiments in which rye has been reported to have been fed to laboratory animals. McCollum, Simmonds, and Parsons (*12*), working with rats, found the proteins of wheat to be superior to the proteins in the other cereals studied and those of barley and rye to be superior to the proteins in corn and oats. Osborne and Mendel (*19*) concluded that in general the proteins of these four cereals vary but little in their ability to promote growth for periods of several months, although for longer periods there was an unusually high mortality of the rats receiving the rye diets. Suzuki, Matsuyama, and Hashimoto (*23*) have reported results somewhat analogous to those of Osborne and Mendel, for they likewise observed a high mortality of rats fed rye at various levels, as compared with rats fed barley and oats.

DIETS USED

The studies reported in this paper were conducted in order to arrive at a better understanding of the cause of the retarded growth of swine receiving rye as the grain part of the ration. The rats used for these experiments were fed individually and, except in the vitamin experiments, were given daily in addition to their basal diets a supplement of 500 mg of yeast tablets that had been immersed in cod-liver oil. In all diets the salt mixture used was that of Hawk and Oser (*5*) (table 1).

TABLE 1.—*Diets used for the determination of the biological values of rye, liver meal, and rye plus liver-meal proteins*

[2 different samples of rye were used, designated rye 1 and rye 2]

Diet 1 (10.1 percent protein)		Diet 2 (10 percent protein) [1]		Diet 3 (10.1 percent protein)		Diet 4 (11.1 percent protein)	
Component	Percent	Component	Percent	Component	Percent	Component	Percent
Rye 1	93.14	Rye 2	91.74	Liver meal	17.19	Rye 1	64.65
Lard	4.41	Dextrin	5.76	Dextrin	71.75	Liver meal	4.82
Salt mixture	2.45	Salt mixture	2.50	Lard	5.16	Dextrin	20.45
				Agar	3.21	Lard	4.72
				Salt mixture	2.29	Agar	.92
						Salt mixture	2.50

[1] Diet 2 was not isodynamic with diet 1.

PROTEIN INVESTIGATION

BIOLOGICAL VALUES OF THE PROTEINS OF RYE, LIVER MEAL, AND RYE PLUS LIVER MEAL

The investigation of the biological values of the proteins of rye and liver meal, separately and combined, was suggested by the successful use of the special liver-meal product as the protein supplement for

rye in experiments with swine by Ferrin and McCarty.[9] The method employed was the usual modification of the method of Osborne, Mendel, and Ferry (*20*). The rye plus liver-meal proteins were given in the proportion that they had been fed to swine, approximately 7 percent of rye protein and 3 percent of liver-meal protein.

Five male rats, weighing 70 g each, were fed on each of the diets given in table 1. Diets 1 and 3, which contained 10.1 percent protein, were made isodynamic by suitable adjustment of their fat content. Diet 4 contained 11.1 percent protein.

The rye contained only a small amount of ergot, which could not be removed because the rye had previously been ground.

In a second experiment rye from another supply was fed as the sole source of protein at a 10-percent protein level in a ration called diet 2. Six male rats were used. They were started when weighing approximately 70 g.

The results of these two experiments are tabulated in table 2 and summarized in table 3.

TABLE 2.—*Weights and food consumption (grams) in the determination of the biological value of the proteins of rye and liver meal*

Rat no.	Inital weight	Total food consumed			Weight		
		At 4 weeks	At 6 weeks	At 8 weeks	At 4 weeks	At 6 weeks	At 8 weeks
DIET 1 (RYE 1)							
1	70	314	486	621	153	193	214
2	70	311	485	655	151	188	209
3	69	294	455	616	148	174	201
4	72	247	412	552	139	184	199
5	69	260	417	589	147	186	226
DIET 2 (RYE 2)							
1	72	386	582	.....	159	198	.....
2	71	313	475	.....	143	168	.....
3	70	365	557	.....	153	195	.....
4	69	351	544	.....	141	177	.....
5	69	343	534	.....	141	169	.....
6	72	298	473	.....	145	167	.....
DIET 3 (LIVER MEAL)							
1	71	254	412	563	129	163	180
2	71	198	331	475	113	142	173
3	69	238	401	532	107	151	181
4	69	223	327	482	113	134	161
5	69	136	250	407	93	137	153
DIET 4 (RYE 1 PLUS LIVER MEAL)							
1	70	281	436	614	141	172	211
2	69	312	502	692	153	200	264
3	69	349	548	750	163	205	228
4	70	289	457	624	133	177	221
5	70	318	520	647	145	198	234

[9] FERRIN, E. F., and MCCARTY, M. A. See footnote 7.

TABLE 3.—*The biological values of the proteins of rye, liver meal, and rye plus liver meal in diets 1 to 4*

Ration tested	Actual biological value [1]			Biological value per 100 g of body weight [2]			Expected biological value			Expected biological value per 100 g of body weight		
	At 4 weeks	At 6 weeks	At 8 weeks	At 4 weeks	At 6 weeks	At 8 weeks	At 4 weeks	At 6 weeks	At 8 weeks	At 4 weeks	At 6 weeks	At 8 weeks
Diet 1 (rye 1)	2.68	2.51	2.27	2.46	1.97	1.62						
Diet 2 (rye 2)	2.23	2.05		2.05	1.64							
Diet 3 (liver meal)	1.76	1.97	1.81	1.95	1.83	1.51						
Diet 4 (rye 1 plus liver meal)	2.45	2.41	2.39	2.26	1.85	1.59	2.40	2.34	2.13	2.33	1.93	1.59

[1] Grams of gain per gram of protein eaten.
[2] Mean weight for experimental period.

That the proteins of rye are very efficient for growth is shown by the high biological values of rye 1 not only for the period of 4 weeks but for 8 weeks as well. The lower biological values for rye 2 can be explained by the fact that while the gain in weight for both the 4- and 6-week periods was approximately the same as for rye 1, the food intake for the 4-week period was 14.3 g and for the 6-week period 12.8 g greater than for the corresponding periods for rye 1.

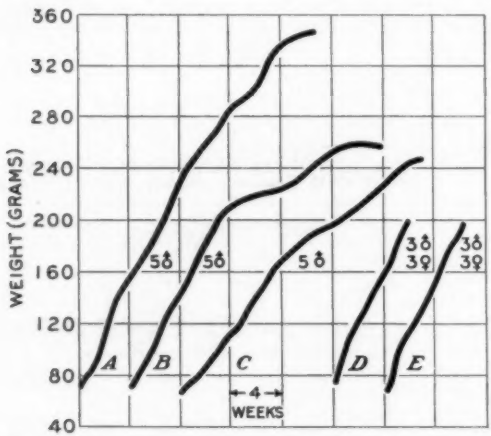

FIGURE 1.—Composite growth curves of rats: *A*, Rye plus liver meal; *B*, rye; *C*, liver meal, all three diets containing 10 percent protein; *D*, rye plus liver meal; *E*, rye plus muscle meat meal, these two diets containing 13.9 percent protein.

When the gain per gram of protein eaten for rye (diet 1) is compared with the gains for rye plus the liver meal (diet 4), it shows that only for the period of 8 weeks was there apparently a slight supplementary relationship between the proteins of rye and liver meal. When, however, the biological values are calculated per 100 g of body weight, there is no indication of such a relationship. In a similar way the actual biological value of the rye plus the liver meal (diet 4) at 8 weeks is apparently greater than the expected biological value for this period, but this difference likewise disappears when both are expressed as a function of body weight.

Because of the slow growth and high mortality occurring in experiments with rats fed rye for comparatively long periods, reported by Osborne and Mendel (*19*) and Suzuki, Matsuyama, and Hashimoto (*23*), the rats were continued on the three diets for 19 weeks.

The growth curves for these rats, given in figure 1, point to the good growth of the rats fed rye and also to the excellent growth of the rats

fed rye supplemented with the liver meal. The growth of the latter is much superior to the former after 8 weeks. This does not necessarily mean that this growth is entirely the result of the supplementary relationship between the proteins of rye and the liver meal, for the animals fed the latter diet continued to eat much more feed during the last part of the experiment. In fact, the average weekly food intake for the last 11 weeks of the experiment was 85.07 g for group 1 (fig. 1, *A*) and 98.56 for group 3 (fig. 1, *B*). One rat in group 3 died from pneumonia at 14 weeks; otherwise there was no mortality of the rats on any of the diets.

A COMPARISON OF THE GROWTH-PROMOTING VALUE OF A LIVER MEAL AND A MUSCLE-MEAT MEAL AS PROTEIN SUPPLEMENTS IN A RYE DIET

Because of the excellent growth over long periods when rye was supplemented with liver meal, it was desired to determine whether or not any other meat product would have an effect similar to liver when fed with rye. Accordingly, a special meat-meal product was obtained to compare with the liver meal. This meat product was prepared in the same manner as the liver meal and was made entirely from beef-muscle tissue.

The two products were fed at 13.92 percent protein level to two groups of rats in isodynamic diets (table 4) in which the proportion between the liver meal or the muscle-meat meal and rye was 3:7, the proportion that had been fed to hogs in the rye-liver meal tests. Three male and three female rats were fed on each diet.

TABLE 4.—*Diets used in the comparison of a muscle-meat meal and a liver meal*

Diet of group 1		Diet of group 2	
Component	Percent	Component	Percent
Rye	90.00	Rye	90.00
Liver meal	6.32	Muscle-meat meal	5.78
Dextrin	1.00	Dextrin	.75
Salt mixture	2.68	Agar	.79
		Salt mixture	2.68

The growth curves in figure 1, *D* and *E*, indicate that the two products were similar in promoting growth. In fact the average weekly food intake and gain was 98.3 g of food and 22.08 g of gain for group 1 (fig. 1, *D*) and 90.6 g of food and 21.75 g of gain for group 2 (fig. 1, *E*). It can be concluded from this evidence that there is nothing specific in the liver meal employed which enhances the feeding value of rye when rye and the liver-meal product are combined in a ration fed to rats.

A PAIRED EXPERIMENT WITH RYE AND RYE PLUS LIVER MEAL AT A 10-PERCENT PROTEIN LEVEL

If there are supplementary relationships between the proteins of rye and the liver meal, they should be in evidence when suitable diets are fed in the same amounts to comparable animals. Accord-

ingly, six pairs of rats were allotted according to Mitchell and Beadles' (*16*) paired-feeding method and fed the two diets shown in table 5. These diets contained 10 percent protein and were equal in energy value. The experiment was continued 10 weeks.

TABLE 5.—*Diets used for the comparison of the proteins of rye and rye plus liver meal at a 10-percent level*

Rye diet		Rye plus liver-meal diet	
Component	Percent	Component	Percent
Rye	91.74	Rye	63.20
Lard	5.26	Liver meal	4.78
Salt mixture	2.50	Dextrin	25.91
		Lard	2.90
		Salt mixture	2.50
		Agar	.71

When the food intake of each pair of rats was equalized, the growth-promoting value of each diet was essentially the same, as is shown in table 6. This indicates that the greater growth after 4 weeks of the rats receiving rye supplemented with the liver meal in experiment 1 was due to the appetizing effect of the liver meal.

TABLE 6.—*Comparative growth-promoting value of rye and rye plus liver meal in paired feeding tests*

[Weights in grams]

Rat number	Sex	Ration	Total food consumed	Total gain in weight	Difference in gain between pair mates [1]
1	♀	Rye	564	116	−6
2	♀	Rye plus liver meal	564	110	
3	♂	Rye	616	140	−6
4	♂	Rye plus liver meal	616	134	
5	♀	Rye	504	101	−8
6	♀	Rye plus liver meal	504	93	
7	♀	Rye	557	99	0
8	♀	Rye plus liver meal	557	99	
9	♀	Rye	544	81	+3
10	♀	Rye plus liver meal	544	84	
11	♂	Rye	546	121	+1
12	♂	Rye plus liver meal	546	122	

[1] + indicates that the difference favors the rat receiving rye plus liver meal; − indicates that the difference favors the rat receiving rye.

A STUDY OF THE LIMITING AMINO ACIDS IN RYE

The experimental data thus far reported point to a relatively high biological value of the proteins of rye. Since there is no report in the literature concerning the amino acid deficiencies of rye protein, this phase of the protein investigation was next undertaken.

The limiting amino acids of rye were investigated by using Mitchell and Beadles' (*16*) paired-feeding method. Ten pairs of rats were used with each of the two amino acids fed, namely, tryptophane and lysine. The lysine was in the form of lysine dihydrochloride.

One rat of each pair received the basal diet containing 8 percent protein, and its pair mate received the basal diet containing 0.25

percent of the amino acid which it was desired to study, the amino acid replacing an equivalent weight of dextrin. The 0.25 percent of lysine dihydrochloride was equivalent to 0.15 percent of lysine. The basal diet consisted of 49.26 percent rye, 10 percent lard, 36.74 percent dextrin, and 4 percent salt mixture.

The average differences in total gains between pair mates on the supplemented and nonsupplemented diets at the end of the consecutive weeks as the experiment progressed are given in table 7.

TABLE 7.—*Average differences in total gain (grams) at weekly intervals between pair mates of 10 pairs of rats fed rye or rye plus tryptophane and lysine dihydrochloride* [1]

Weeks (number)	Difference in gain due to 0.25 percent of tryptophane	Difference in gain due to 0.25 percent of lysine di-HCl	Weeks (number)	Difference in gain due to 0.25 percent of tryptophane	Difference in gain due to 0.25 percent of lysine di-HCl
1	−0.4	+0.8	4	−0.1	+3.5
2	+.15	+1.6	5	.0	+6.18
3	−.1	+1.9	6	−1.4	+7.75

[1] + indicates that the difference favors the rat receiving the supplemented diet; − indicates that the difference favors the rat receiving the nonsupplemented diet.

A valid criticism of this experiment, as well as of similar experiments of other investigators, is that the interpretation of the results is necessarily modified by the amount of the limiting amino acids being studied which were present in the yeast supplement. It may be calculated from the amino acid distribution in yeast protein, as given by Meisenheimer (*13*), and the protein content of dry yeast (which was employed in the experiment here reported), as given by Hawk, Smith, and Holder (*6*), that dry yeast contains 5.241 percent lysine and 0.262 percent tryptophane. Therefore, the lysine furnished by the yeast in this experiment amounted to approximately two and one-half times that provided in the lysine dihydrochloride fed as 0.25 percent of the diet. The tryptophane furnished by the yeast protein amounted to 7.88 percent of that fed as 0.25 percent of the diet.

That tryptophane is not a limiting amino acid in rye is indicated by the fact that at the end of 7 weeks the average difference in total gain between pair mates of 10 pairs of rats was 1.4 g, in favor of the control. Lysine, however, would seem to be a limiting amino acid of rye when rye furnishes protein in the basal diet at an 8-percent level. As may be observed in table 3, the average difference in growth between pair mates of 10 pairs of rats at the end of 6 weeks favored the experimental animal by 7.75 g. Furthermore, the gain in weight which favored the experimental animal the first week became progressively greater each week that the experiment continued.

The statistical data showing the significance of the average differences between pair mates in the tryptophane and lysine experiments are given in table 8.

TABLE 8.—*Statistical results of paired feeding experiments with rye at an 8-percent protein level plus tryptophane and lysine* [1]

Item compared	Tryptophane	Lysine
Mean difference in total gain in weight.....grams..	−1.6	+7.66
Standard deviation	5.10	3.75
Z value	.31	2.04
Probability	.81077	.99978

[1] Student (*1*).

The mean differences favoring the controls in total gains between pair mates in the tryptophane experiment show conclusively that this amino acid had no supplementing effect when fed with rye. The yeast supplement, although furnishing a small amount of tryptophane, probably did not supply enough to render this conclusion invalid. In the lysine experiment there is a probability of 0.99978, i.e., odds of over 4,000 to 1, that the mean difference of 7.65 g was due to the supplementing effect of the lysine. This is especially noteworthy in view of the large amount of lysine furnished in the yeast.

From the results of this phase of the study it can be concluded that rye proteins are comparable in quality to the proteins of corn, wheat, and oats, since Mitchell and Smuts (*17*) found that the proteins of these cereals are deficient in lysine. They found corn to be deficient in tryptophane and oats to be probably deficient in some other amino acid.

POSSIBLE DEFICIENCIES OF VITAMINS B AND G IN RYE

The possible deficiencies of rye in vitamins B and G were next investigated. No attempt, however, was made to make a comprehensive assay of rye for these vitamins.

Rye was fed at 14-, 27-, 40-, 53-, and 66-percent levels. It was incorporated into the basal diet by replacing an equivalent weight of dextrin. The basal diet consisted of purified casein, 20 percent; dextrin, 66 percent; lard, 10 percent, and salt mixture, 4 percent.

Six rats, one female and five males, 28 days of age, were fed on each of the experimental diets. Each group was first given the basal diet for 16 days to deplete their bodies of the vitamin to be studied. The rats that were used for the vitamin G study were given daily, in addition to the basal diet and the experimental diets, 0.5 g of tikitiki tablets that had been immersed in cod-liver oil, while those used for the vitamin B study were given daily 0.5 g of autoclaved yeast that had been immersed in cod-liver oil. This yeast was autoclaved for 6 hours at 15 pounds pressure. The tikitiki was prepared in the following manner. Eight hundred grams of ether-extracted rice polish was placed in a large flask and heated at 50° to 60° C. for 3 hours with 4 l of 80-percent alcohol. The alcohol extract was recovered after filtering off the extracted rice polish with a Buchner funnel. The same procedure was repeated with 3 l of alcohol and finally with 2 l. At this time the entire volume of alcohol remaining from the original 9 l was placed in a large vessel and allowed to evaporate to a volume of about 1 l in a partly open drying drawer. It was then evaporated upon 250 g of dextrin. After drying, the equivalent of the preparation in terms of rice polish was calculated. This was done in order that in each preparation an amount of the tikitiki might be fed so as to insure an adequate supply of vitamin B.

The growth curves in figure 2 of the rats used in this investigation show that only on the higher levels of rye was there a sufficient amount of either vitamin for maximum growth. Since entirely satisfactory growth resulted at the 66-percent level, it seems reasonable to assume that a deficiency of these vitamins undoubtedly does not occur when rye is fed to swine as 80 percent or more of the ration.

An inspection of the growth curves in figure 2 does not indicate that rye furnishes appreciably more of the vitamin B than of the vitamin G requirement for growth at the several levels tested. The greater growth at the 27-percent level in the G assay than in the B assay at this level is explained entirely by greater average food intake of 77 g per rat in the former. Difference in food intake explains in part the greater growth at the 66-percent level in the B assay than in the G assay at this level. It seems improbable, therefore, that failure in growth when rye is fed at high levels can be attributed to a deficiency of either vitamins B or G. That rye does not furnish appreciably more of the vitamin B than of the vitamin G requirement for growth is not in accordance with the findings of Hetler, Meyer, and Hussemann (*8*) in regard to the vitamin B and G content of oats, corn, and wheat. These investigators found that a diet containing 50 percent of any of the cereals would provide enough vitamin G for approximately normal growth and that 25 percent of any of the cereals would supply sufficient vitamin B.

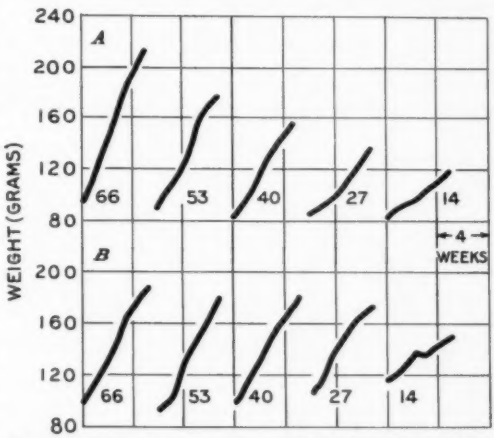

FIGURE 2.—Composite growth curves of 1 female and 5 male rats on each of various indicated percentage levels of rye as a part of a purified diet in a study of the possible deficiencies of rye in vitamins B and G. Each rat in the vitamin B assay (*A*) received a daily supplement of a 0.5 g tablet of autoclaved yeast. Each rat in the vitamin G assay (*B*) received a daily supplement of a 0.5 g tablet of tikitiki evaporated on dextrin. Each tablet was saturated with cod-liver oil by immersion before feeding.

In order to investigate the possibility of vitamin G being present in the tikitiki that was being used, 14 rats were fed the purified basal diet for 2 weeks with a daily supplement of 0.5 g of dextrin tablets that had been immersed in cod-liver oil. At the end of this period, at which time growth had stopped, 0.5 g of tikitiki evaporated on dextrin was substituted for the dextrin. The composite growth curve of the 14 rats is given in figure 3, *I*. It shows that growth was resumed, although at a slow rate, after the diet was supplemented with tikitiki. This offers evidence that at least a part of the growth in the vitamin G assay was very likely the result of the presence of vitamin G in the tikitiki. Had the tikitiki employed been free from vitamin G, it is possible that the results of the comparative B and G assays shown in figure 2 would have been more in accord with those of Hetler et al., (*8*) for the cereals which they studied.

To test this possibility, rye was fed at 20-, 30-, and 35-percent levels with and without autoclaved yeast tablets that had been immersed in cod-liver oil. The resulting growth, as shown in figure 3, *A* to *H*, demonstrates quite conclusively that at the levels at which the rye was fed, vitamin G and not vitamin B was the limiting factor for growth. It is surprising that growth was better at the 35-percent level with autoclaved yeast than in the former experiment at the 53-percent level. This may have been due, in part, to the fact that there was no depletion period for the experiments shown in figure 3. However, the rye fed in the two experiments was not from the same source. It may well be that rye varies in its vitamin content depending upon the variety, climatic and storage conditions, and other environmental factors.

NUTRITIVE VALUE OF THE RYE GERM

Mellanby (*15*) has reported that dogs fed diets deficient in fat-soluble vitamins and containing wheat germ developed severe incoordination, muscular weakness, and spinal-cord degeneration. In an earlier report of this work Mellanby (*14*) mentions that his dogs were quite variable in response to such diets; some of his experimental animals were never affected. Since the condition was similar to that found in human beings dying from ergotism, Mellanby (*15*) investigated the effect of ergot upon the nervous system of puppies and found that the same degenerative changes of the spinal cord were produced. This was the case, however, only if the diet was devoid of fat-soluble vitamins. The addition of irradiated ergosterol did not prevent the degeneration, while the addition of butter, cabbage, or egg yolk did: hence the conclusion that vitamin A was the protective factor. He found also that rye germ and sometimes even white flour would produce the condition. He reported that it was difficult to produce the degeneration in animals whose diet had been rich in vitamin A before the experimental feeding began.

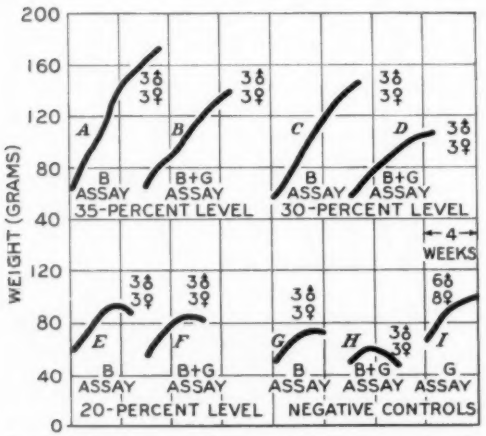

FIGURE 3.—Composite growth curves of rats in an assay of rye for vitamins B and G. In the vitamin B assay each rat received a daily supplement of a 0.5 g tablet of autoclaved yeast. In the vitamin B and G assay each rat received a daily supplement of a 0.5 g tablet of dextrin. In the vitamin G assay (negative controls) each rat received a daily supplement of a 0.5 g tablet of tikitiki evaporated on dextrin. Each tablet was saturated with cod-liver oil by immersion before feeding. Rye levels are indicated in percentage.

It is not clear from the report of these experiments whether or not the absence of vitamin A in noncereal diets produces these changes.

In direct contradiction to the experiments of Mellanby (*15*) is the work of Suzman, Muller, and Ungley (*22*). They were unable to

produce spinal-cord degeneration in dogs fed a high cereal diet deficient in vitamin A. A syndrome of anemia, skin lesions, anorexia, and changes in the concentration of the blood lipoids developed irrespective of whether or not the dogs were receiving vitamin A. These writers felt this to be due to a lack of one or more components of the vitamin B complex other than the antineuritic factor.

Kling (*10*) found that pigs fed for 6 weeks on rye germ became sick and some died, although it was not known definitely whether or not the trouble was due to the rye germ.

Because of this report and the reports of Mellanby (*15*) in which rye germ had produced spinal-cord degeneration, it seemed that the cause of the unsatisfactory results when rye is fed to swine might possibly reside in the rye germ. Accordingly, some rye germ was obtained[10] and fed to rats.

Six rats, three males and three females, were fed the regular purified diet. A comparable group of rats received the basal diet with 10 percent rye germ replacing an equivalent weight of dextrin. A third group received the basal diet in which 15 parts of dextrin had been replaced by 10 parts of rye germ and 5 parts of the liver meal which was employed in the protein studies. These diets are given in table 9.

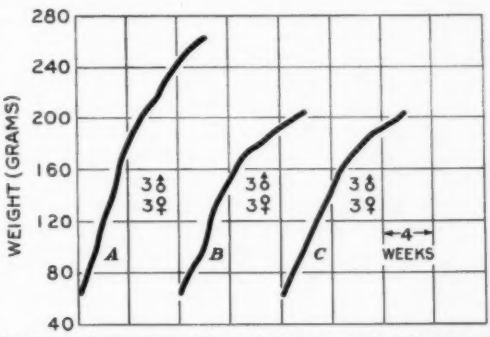

FIGURE 4.—Composite growth curves of rats in a study of the effect of rye germ upon growth: *A*, Purified basal diet with 10 percent rye germ and 5 percent liver meal (diet 3); *B*, purified basal diet with 10 percent rye germ (diet 2); *C*, purified basal diet (diet 1).

The rye germ fed as 10 percent of the diet did not retard growth as is shown by the composite growth curves in figure 4, *B* and *C*. Neither did it have any effect upon food consumption, for the average weekly food intake of the rats receiving the purified diet was 53.4 g, as compared with 53.7 g for the rats receiving the purified diet plus the 10 percent of rye germ. The growth curves also reveal the favorable effect of the 5 percent of liver meal upon growth (fig. 4, *A*). This greater growth, however, was the result of appetite stimulation, for the rats receiving diet 3, containing the liver meal, ate 66.53 g of food per week as compared with the 53.74 g eaten by the rats receiving diet 2.

One rat on the purified diet containing 10 percent rye germ died at the end of 7 weeks. Its death was thought to have been caused by the extremely hot weather prevailing at that time. The fact that the growth rate of the majority of the rats on all three diets was materially lessened at the time substantiates this conclusion.

In view of the results of these experiments the conclusion seems valid that there is nothing in rye germ when fed in a complete diet that is detrimental in any way to rats.

[10] The rye germ employed was furnished by General Mills, Inc., of Minneapolis, through the courtesy of Dr. C. H. Bailey, of the Minnesota Agricultural Experiment Station, whose kindness is gratefully acknowledged.

TABLE 9.—*Diets used to determine the effects of rye germ upon growth and food consumption when fed to rats*

Diet 1		Diet 2		Diet 3	
Component	Percent	Component	Percent	Component	Percent
Dextrin	66	Rye germ	10	Rye germ	10
Casein	20	Dextrin	56	Liver meal	5
Lard	10	Casein	20	Dextrin	51
Salts	4	Lard	10	Casein	20
		Salts	4	Lard	10
				Salts	4

SUMMARY AND CONCLUSIONS

The biological value of the proteins of rye was investigated by feeding rats rye, a liver-meal product, and rye plus the liver meal in diets at a 10-percent protein level. A special meat meal, made from beef-muscle tissue, was fed to determine whether any other meat product would have an effect similar to the liver meal when fed with rye. The amino acid deficiencies of rye were studied by using Mitchell and Beadles' (*16*) paired-feeding method. Rye, at an 8-percent protein level, was used as part of the basal diet. Two amino acids, lysine dihydrochloride and tryptophane, were fed as 0.25 percent of this diet.

The possible deficiencies of vitamins B and G in rye were investigated by feeding rye at various levels as parts of a purified basal diet.

Rye germ, considered toxic by one investigator (*15*), was fed as 10 percent of a purified diet to determine its nutritive value.

From these experiments the following conclusions have been drawn:

The favorable effects on growth of rats of supplementing rye rations with a special liver meal are not attributable to an enhancement of the biological value of the protein mixture.

Muscle-meat meal, when fed with rye, has a favorable effect on growth analogous to that obtained with liver meal.

Lysine, but not tryptophane, is the first limiting amino acid of the proteins of rye.

The study of the possible deficiencies of vitamins B and G in rye shows that it is very unlikely that a deficiency of either vitamin will occur at the level at which rye is fed in livestock rations.

Rye germ, when fed as 10 percent of a complete diet, does not retard the food intake or growth of rats. It seems unlikely that unsatisfactory results with rye as a feed for growing, fattening swine are attributable to the germ fraction of the rye.

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RESISTANCE TO COMMON BEAN MOSAIC IN THE GREAT NORTHERN FIELD BEAN [1]

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INTRODUCTION

Certain varieties of the common bean, *Phaseolus vulgaris* L., have been shown to be resistant to common bean mosaic. Reddick and Stewart [3] demonstrated that the Robust variety is highly resistant or immune to this disease. Two new varieties of stringless Green Refugee, Wisconsin Refugee, and Idaho Refugee developed by Pierce and Walker,[4] were shown by them to be resistant to common bean mosaic.

In a study of varietal susceptibility the writer [5] showed that besides Robust the varieties Corbett Refugee and Great Northern UI No. 1 were immune to infection with the virus of common bean mosaic. Great Northern UI No. 1 was a selection made in 1927 from commercial Great Northern. Commercial strains of Great Northern grown in Idaho prior to the introduction of the UI No. 1 strain were nearly 100 percent susceptible to mosaic.[6] The finding of the resistant UI No. 1 in commercial Great Northern gave impetus to further selection within the commercial strains. A large number of resistant selections differing in certain agronomic characters have been obtained. The results of the resistance and yield trials of nine of these selections are presented in this paper.

MATERIALS AND METHODS

The selections herein reported upon were all made from commercially grown Great Northern beans. Single plant selections of healthy plants were made from fields having a high mosaic infection. Thus the chances of obtaining mosaic-resistant plants were greatly increased. The selections were tested in single-plant units for susceptibility to common bean mosaic in the field and greenhouse. The field trials were made at Twin Falls, Idaho, in an area conducive to the rapid natural spread of bean mosaic. The selections were tested in the greenhouse for resistance to mosaic infection by making artificial inoculations at the time the plants were in the young 2-leaf stage. The entire upper surfaces of these young leaves were rubbed

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[2] The author acknowledges his indebtedness to Dr. C. W. Hungerford for valuable suggestions offered in the course of these investigations, and to J. M. Raeder and E. J. Wellhousen for the execution of the field trials in 1931.

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with leaf material infected with mosaic. This method of inoculation gave between 80 and 100 percent mosaic infection on susceptible varieties. Selections which developed no symptoms of mosaic were tested for the presence of the virus by making inoculations to a known susceptible variety.

RESULTS

FIELD TRIALS OF RESISTANCE

In the field trials a large number of selections have been made, many of which possessed resistance to mosaic; however, the results from only nine of these and a commercial sample of Great Northern are included (table 1). These strains have given the most consistently high performance in yield. They also give a general cross section of the various types of beans to be found in commercial Great Northern.

TABLE 1.—*Summary of yield, season, and mosaic-resistance trials of Great Northern bean selections grown at Twin Falls, Idaho*

Selection	Calculated yield per acre				3-year total stand of plants	Mean percentage of common bean mosaic [3]	Season-days from planting to maturity
	1930 [1]	1931 [1]	1933 [2]	3-year average			
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Number*	*Percent*	*Number*
UI No. 1	42.6	42.3	50.0	45.0	1,516	0	86
UI No. 56	43.5	42.7	48.4	44.9	1,841	0	83
UI No. 59	38.0	46.0	54.0	46.0	1,446	0	85
UI No. 60	[4] 40.9	47.4	51.1	46.5	948	0	85
UI No. 73	46.6	44.0	46.4	45.7	1,582	0	86
UI No. 77	38.5	45.0	50.8	44.8	1,831	0	92
UI No. 81	49.7	48.5	46.7	48.3	1,538	0	88
UI No. 95	[4] 35.2	48.5	48.1	43.9	1,129	0	85
UI No. 123	[4] 44.0	43.3	46.4	44.6	870	0	86
Common Great Northern	24.0	28.1	33.9	28.7	1,566	±95	92

[1] Average of twelve 20-foot row replications.
[2] Average of four 35-foot row replications.
[3] From 2 to 3 percent of all selections were affected with yellow bean mosaic; but only the common Great Northern showed symptoms of common bean mosaic.
[4] Not replicated in 1930.

As shown in table 1, none of the nine selections exhibited symptoms of common bean mosaic, even though grown beside common Great Northern which developed mosaic to as high as 100 percent in some seasons. In figure 1 is shown the resistant UI No. 81 selection and a mosaic-susceptible selection grown in the field plots in 1930. Many of the original selections were found to be susceptible to mosaic and were therefore discarded.

A small percentage of the plants in the various resistant selections showed a yellow type of bean mosaic. This type of mosaic was previously shown [7] to be caused by a virus distinct from that of common bean mosaic. The disease was named yellow bean mosaic and the virus concerned, bean virus 2. Observations and inoculation trials during the 1933 season demonstrated quite conclusively that the small percentage of yellow bean mosaic occurring in the resistant Great Northern strains was directly traceable to mosaic infected sweetclover growing nearby. It had been previously shown [7] that

[7] PIERCE, W. H. See footnote 5.

the yellow bean mosaic virus (bean virus 2) was transmissible to sweetclover.

GREENHOUSE TRIALS OF RESISTANCE

In the greenhouse trials a series consisting of 30 plants of each of the Great Northern strains was artificially inoculated with the virus of common bean mosaic (bean virus 1). Only the common Great Northern strain developed symptoms of common bean mosaic. A test of those strains not showing symptoms was made to determine whether or not they carried the virus of common bean mosaic in a masked condition. Inoculations from 10 individual plants of each strain were made to 5-plant units of a mosaic-susceptible line of Great Northern. In no case was the virus of common bean mosaic found to be present.

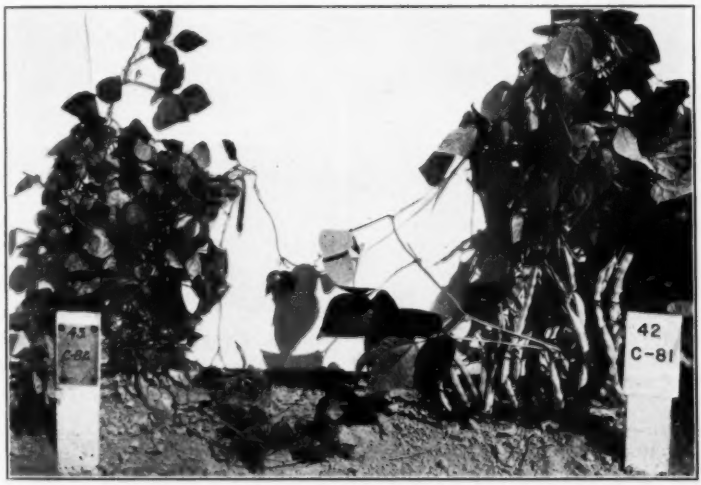

FIGURE 1.—Comparison of two strains of Great Northern beans in the trial plots at Twin Falls, Idaho, 1930. Left, a mosaic-susceptible strain; right, mosaic-resistant strain UI No. 81.

YIELD TRIALS

In 1930 and 1931 the resistant strains were grown in 20-foot rows and replicated 12 times. In 1933 four replications of 35-foot rows were grown. No trials were made in 1932. The yields were recorded in pounds and then calculated in bushels per acre. The mean yield for each strain is given in table 1. The yields of all resistant strains were significantly higher than common Great Northern infected with mosaic. Strain UI No. 81 gave the highest yield of all strains tested. The yields of the other strains (table 1) were closely grouped.

SEASON

The length of time in days from planting to maturity was recorded for each of the strains (table 1). The season varied somewhat each year; but the number of days given in table 1 represents the averages obtained from the 3-year data. Strain UI No. 56 required the shortest season to mature and UI No. 77 the longest. The long season for

common Great Northern was occasioned by the infection with common bean mosaic. This disease delays maturity materially.

PLANT TYPE

Considerable variability in vining habit exists among the different selections. UI No. 1 starts vining early, and the vines attain considerable length, thus causing some inconvenience in harvesting. UI No. 77 is also a heavy viner. UI Nos. 56, 81, and 123 produce a short- to medium-length vine and are the most satisfactory from the standpoint of easy harvesting.

SEED TYPE

The shapes and sizes of seeds varies with the different selections (fig. 2). The seeds of selections UI No. 59, UI No. 73, and UI No. 123 approximate closely the general type of commercial Great Northern. The UI No. 1 strain is more cylindrical and selections UI No. 56, UI No. 81, and UI No. 95 are slightly smaller than common Great Northern. The Great Northern trade seems to favor the larger sized beans.

DISCUSSION

The increasing importance of mosaic as a limiting factor in the production of Great Northern beans in Idaho led to an investigation of the possibility of obtaining strains of this variety resistant to common bean mosaic. In fields showing a very high percentage of infection occasional plants were noted which appeared to be healthy. The selection and testing of such plants showed that while most of them had merely escaped infection some possessed resistance. It was noted early in the work of testing the resistant selections that they were not all alike with respect to season, type of plant, and size and shape of seed.

The Great Northern field bean is believed to be a variety taken over bodily from the Indian agriculture of the Dakotas in about 1886. Only during the last 20 years has the variety reached commercial proportions, and it has undergone relatively little selecting other than natural and machine selection. It is not surprising, therefore, that plants differing in type were found in the old Great Northern variety. Even though the variety as originally grown was somewhat heterogeneous, it should be pointed out that it tended to be made up largely of one type of plant and seed. The resistant variants which have been found accounted for a very small proportion of the total population of the old Great Northern variety.

The mosaic resistance of the different selections appears to be of the same order. The inoculation tests gave no indication that one strain was more resistant than another. All the strains reported were completely resistant. Cross-breeding studies, however, may show differences in the genetic nature of resistance of the various strains. It seems possible, on the other hand, that the mosaic resistance of the various resistant strains may have had a common origin and that the diversity of plant type of the resistant selections may have come about as a result of natural crossing with different susceptible types. However, if such has been the case, the resistant lines appear to have become fixed with respect to resistance and type. No segregation of plant characters was noted in this study, but it should be stated

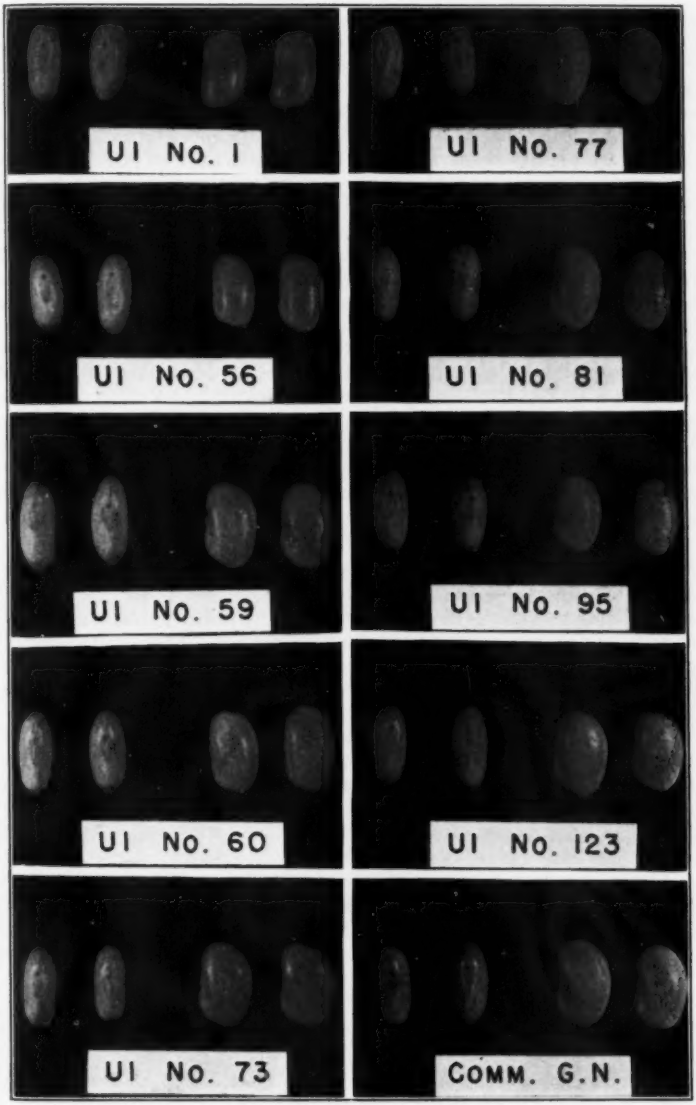

FIGURE 2.—Comparison of seed types of various strains of Great Northern beans; natural size.

that a small percentage of natural crossing is known to occur in beans and that such undoubtedly has occurred somewhere in the history of the various Great Northern strains.

Strains UI No. 1, UI No. 59, and UI No. 81 are now being grown commercially in southern Idaho. These strains, along with the resistant strains selected by growers, have largely supplanted the old type commercial Great Northern which was highly susceptible to mosaic. These strains are not only resistant to common bean mosaic but, as previously shown for the UI No. 1 strain,[8] also possess some tolerance to yellow bean mosaic. Infections of the latter are not easily recognized in plants also affected with common bean mosaic. Thus, in varieties resistant to the common form, infections with the yellow form are quite apparent and easily recognized. This fact should not be allowed to confuse the general understanding of the bean-mosaic problem, for, as pointed out in a previous paper,[8] yellow bean mosaic is much more severe on varieties susceptible to the common form.

Occurrences of yellow bean mosaic are quite common in southern Idaho, especially in plantings surrounded by irrigation ditches or fence rows which harbor sweetclover infected with the virus of yellow bean mosaic (bean virus 2).

The disease has been shown to be not seed borne in the bean.[8] Its occurrence therefore appears to be dependent upon some overwintering host such as sweetclover.

SUMMARY

The data reported in this paper are concerned primarily with the resistance of nine selections of Great Northern beans to the virus of common bean mosaic. The nine selections, representing the higher yielding strains, were all found to be completely resistant to common bean mosaic.

The yields of the resistant strains were significantly higher than the yield of mosaic-susceptible common Great Northern.

Variations in plant type, seed type, and length of growing season were noted for the different selections.

Three of the resistant selections are now being grown commercially in Idaho.

[8] PIERCE, W. H. See footnote 5.

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